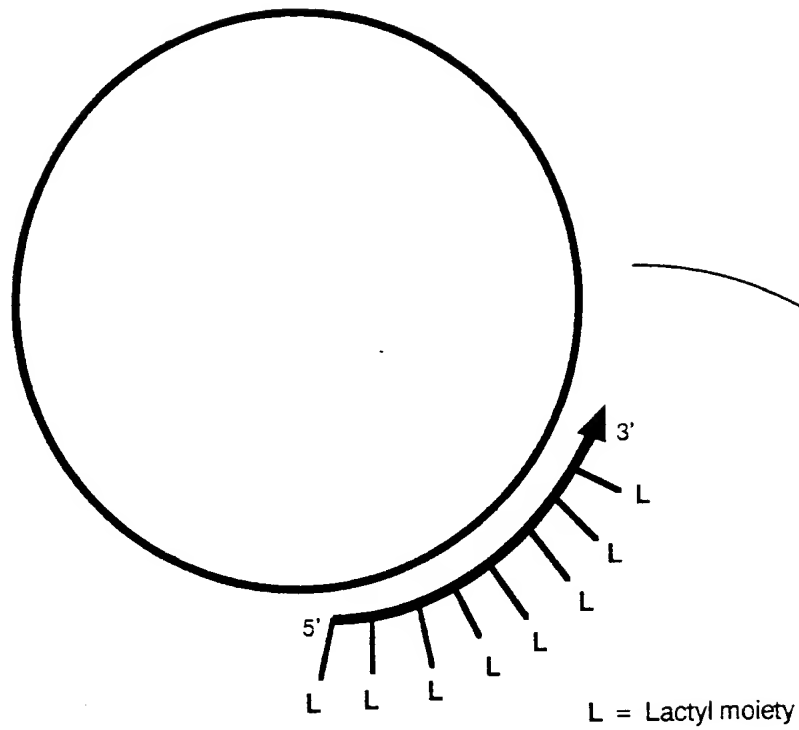


(a)



(b)

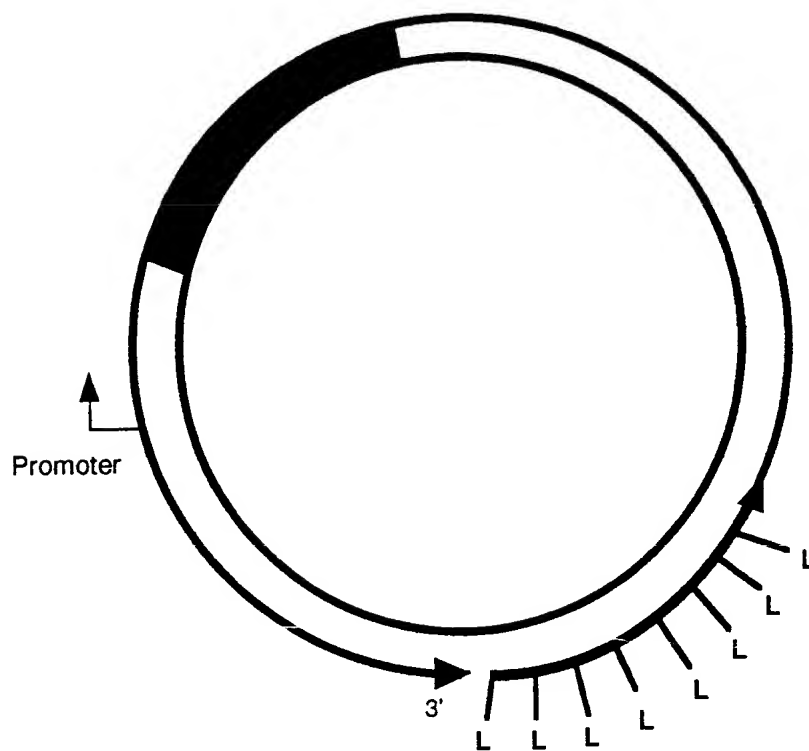


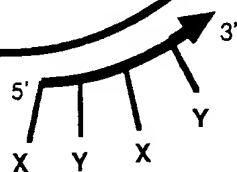
Figure 1

Attachment of Ligands Through Primer Region

(a)

X = Nuclear Localisation Signal

Y = fusogenic peptide



(b)

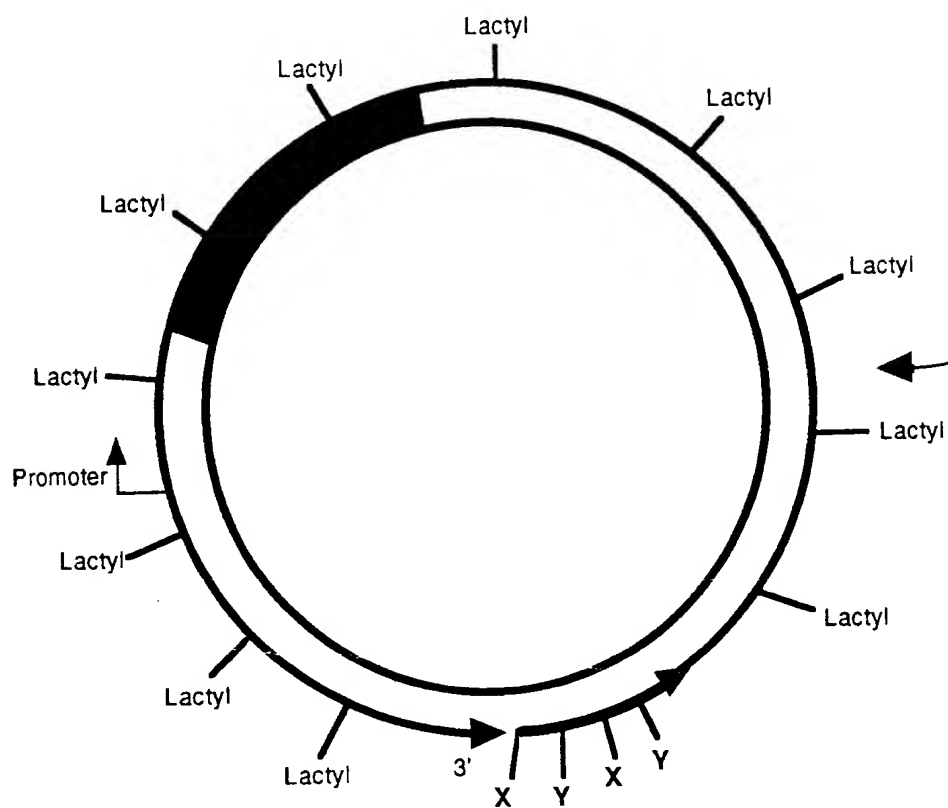


Figure 2
Attachment of Ligands by Incorporation of
Modified Nucleotide Precursors

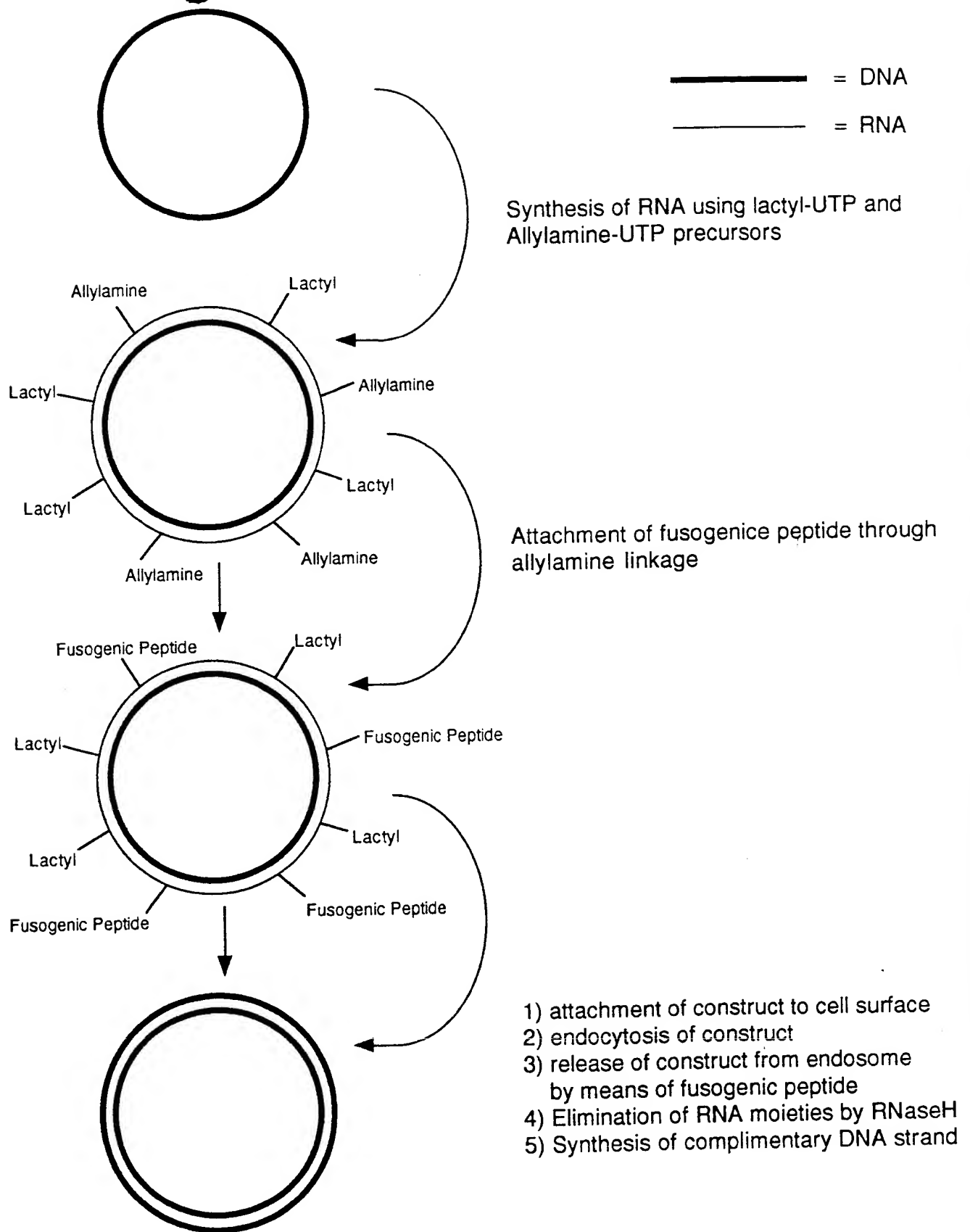


Figure 3
Incorporation of Ligands through Modified Ribonucleotides

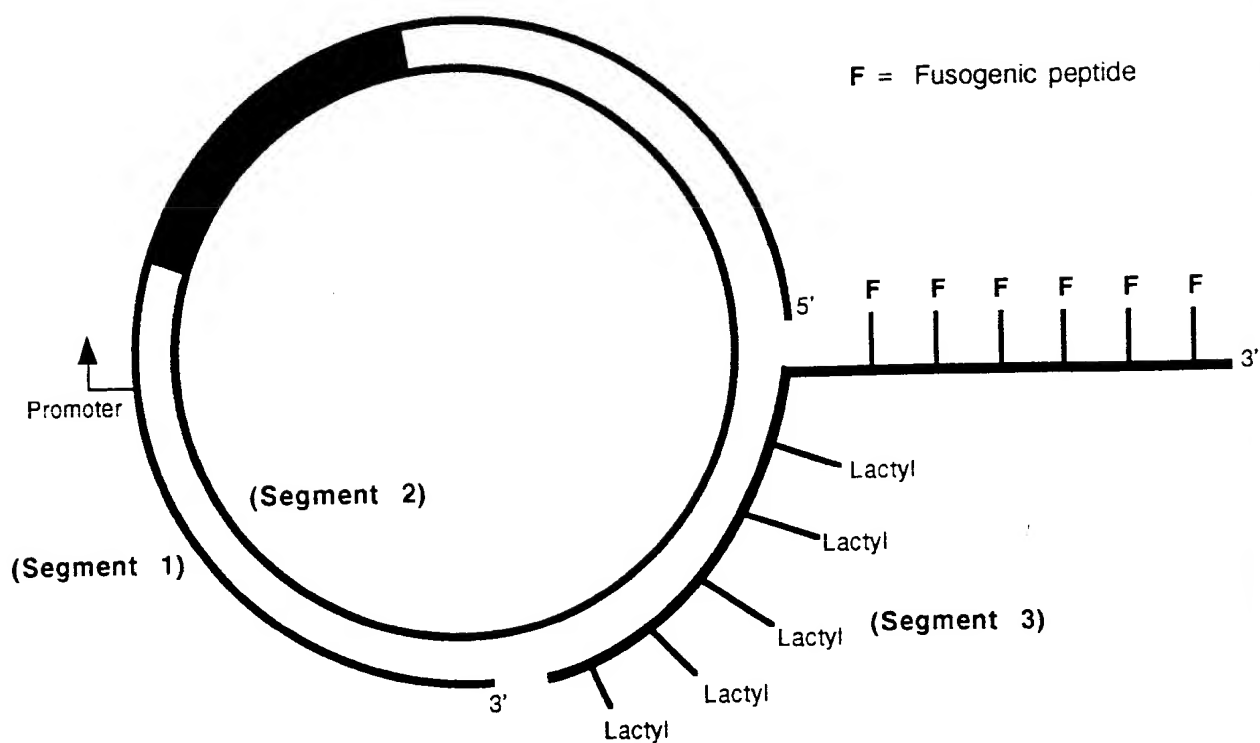


Figure 4

Attachment of Ligands through a 3' tail

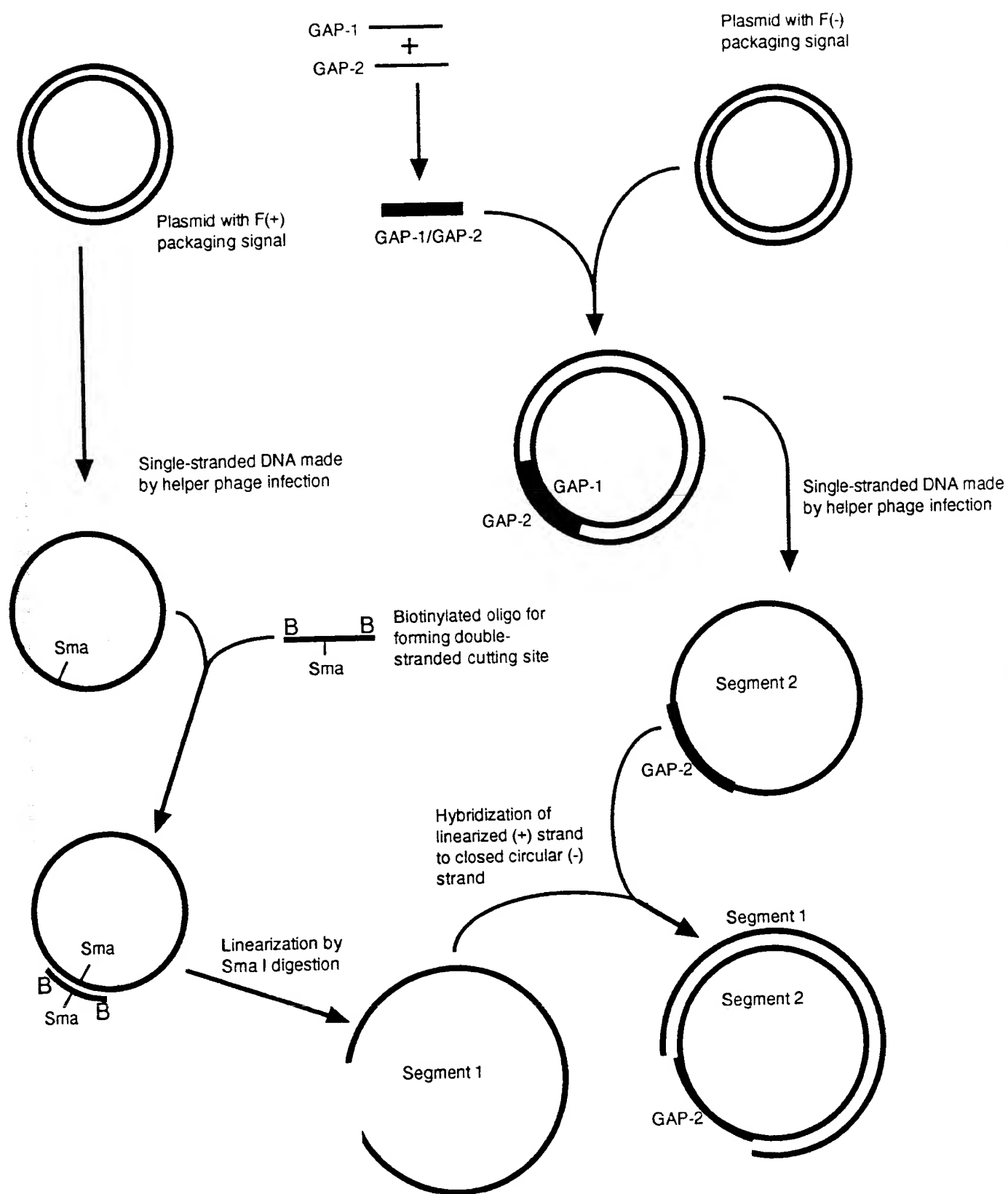
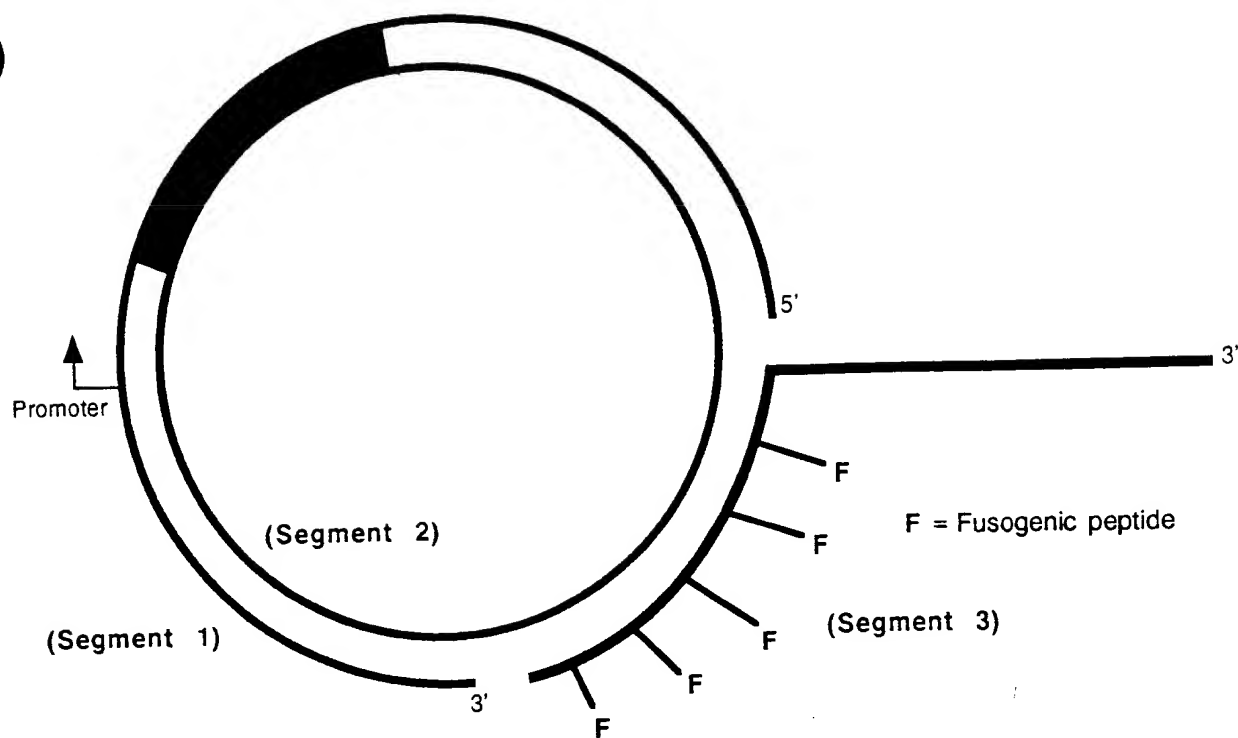
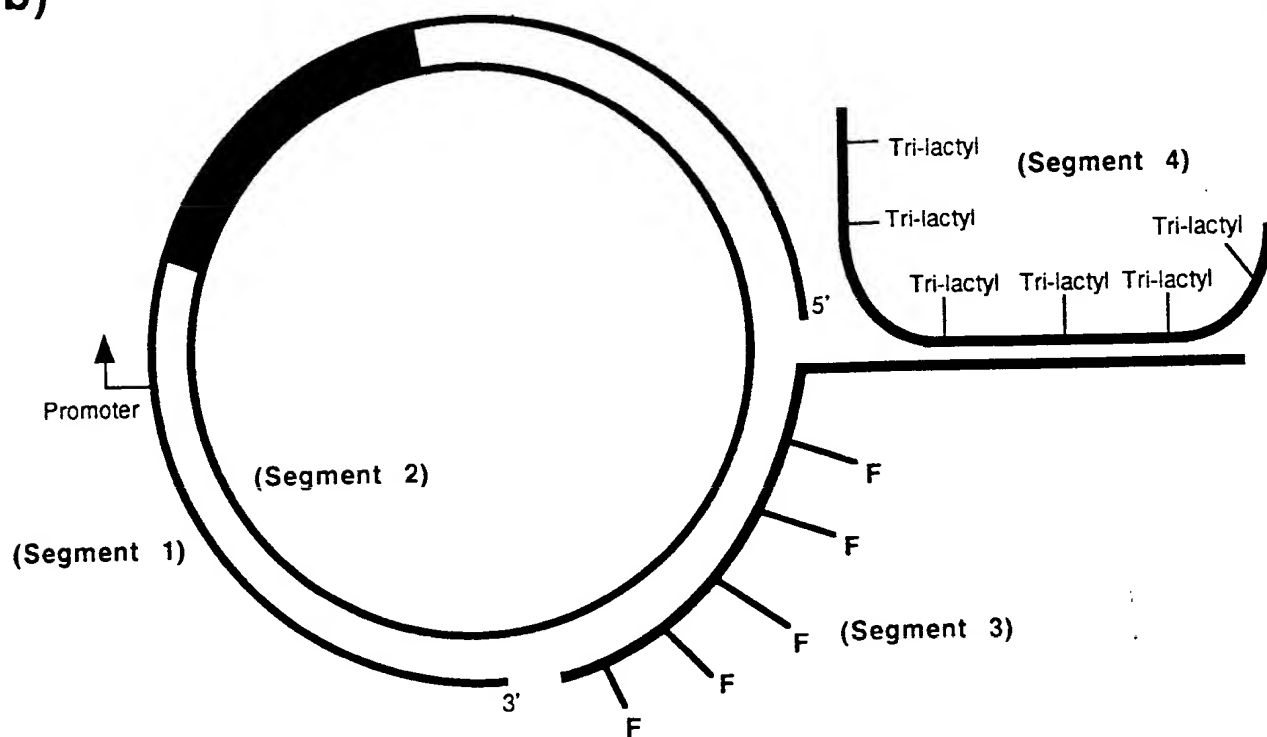


Figure 5
Preparation of Gapped Circle

(a)



(b)

**Figure 6**

Attachment of Ligands through hybridization to a 3' tail

— = DNA
 — = RNA

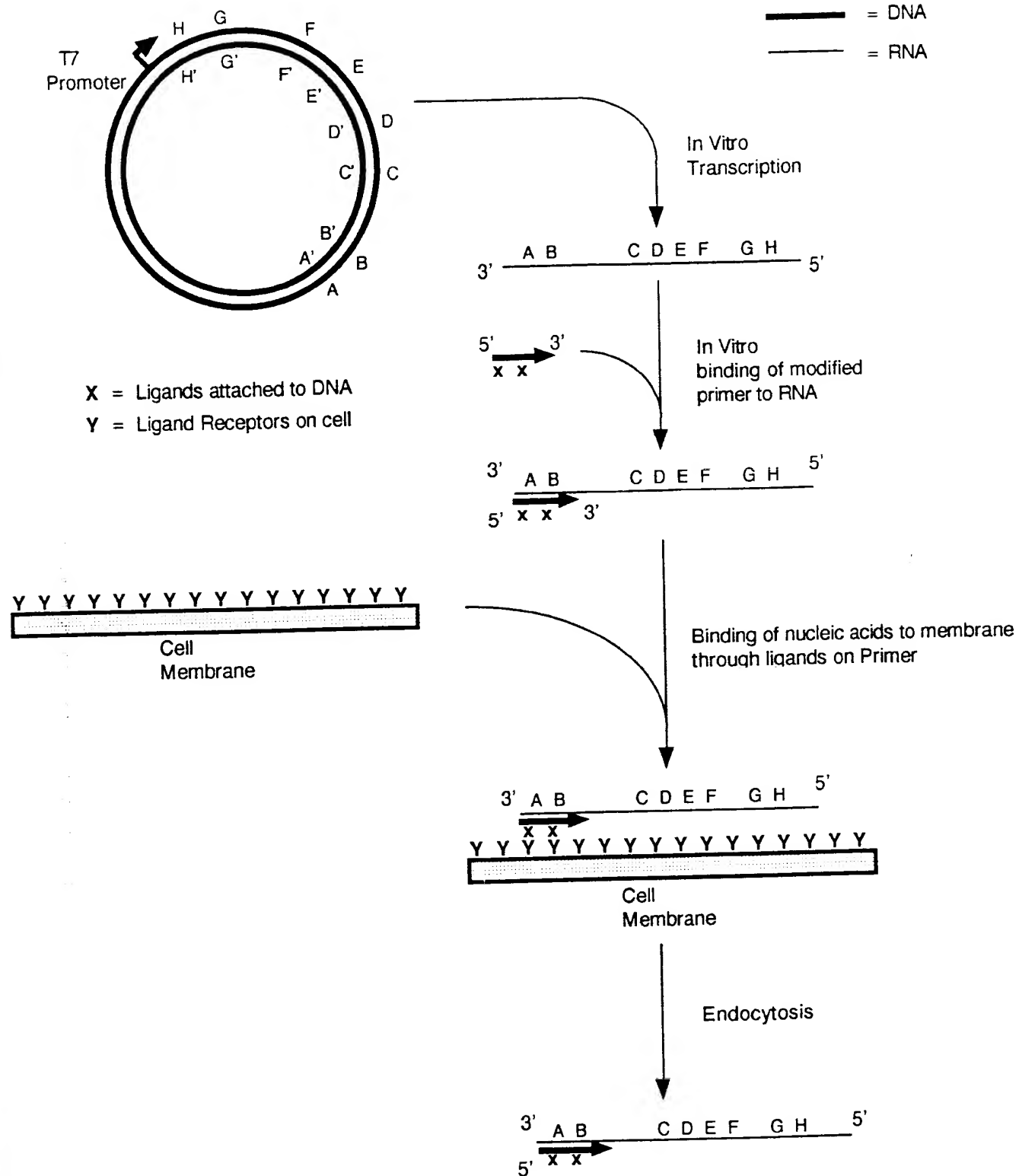


Figure 7
 RNA with Ligands on Primer

(Continued in Figure 8)

Continued from Figure 7

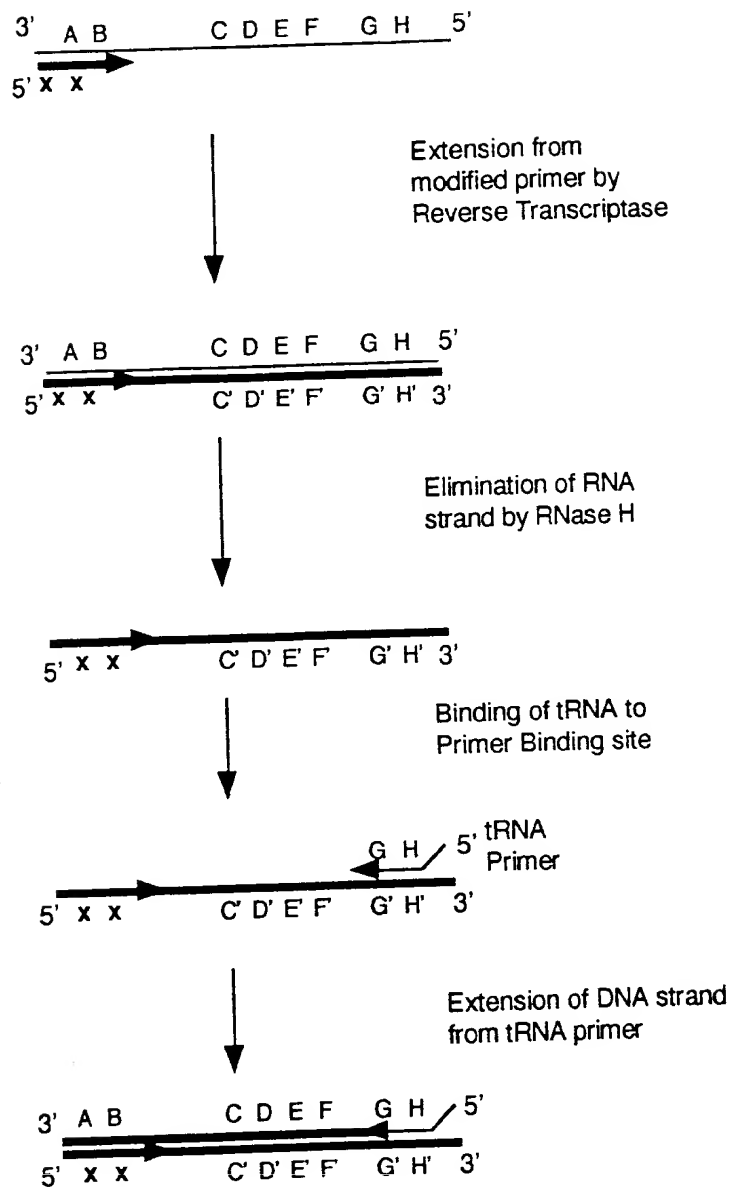
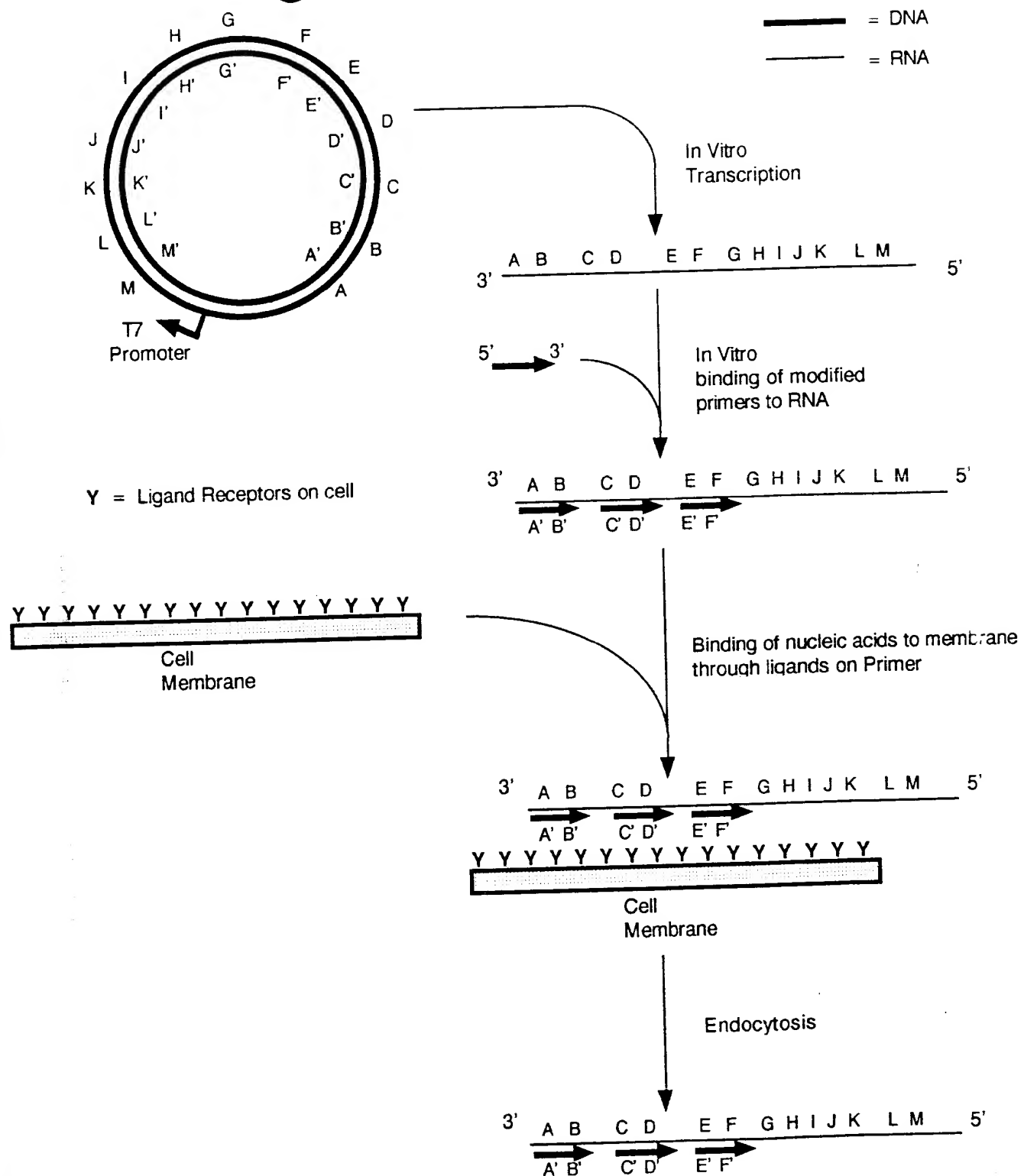


Figure 8
RNA with Ligands on Primer (Continued)

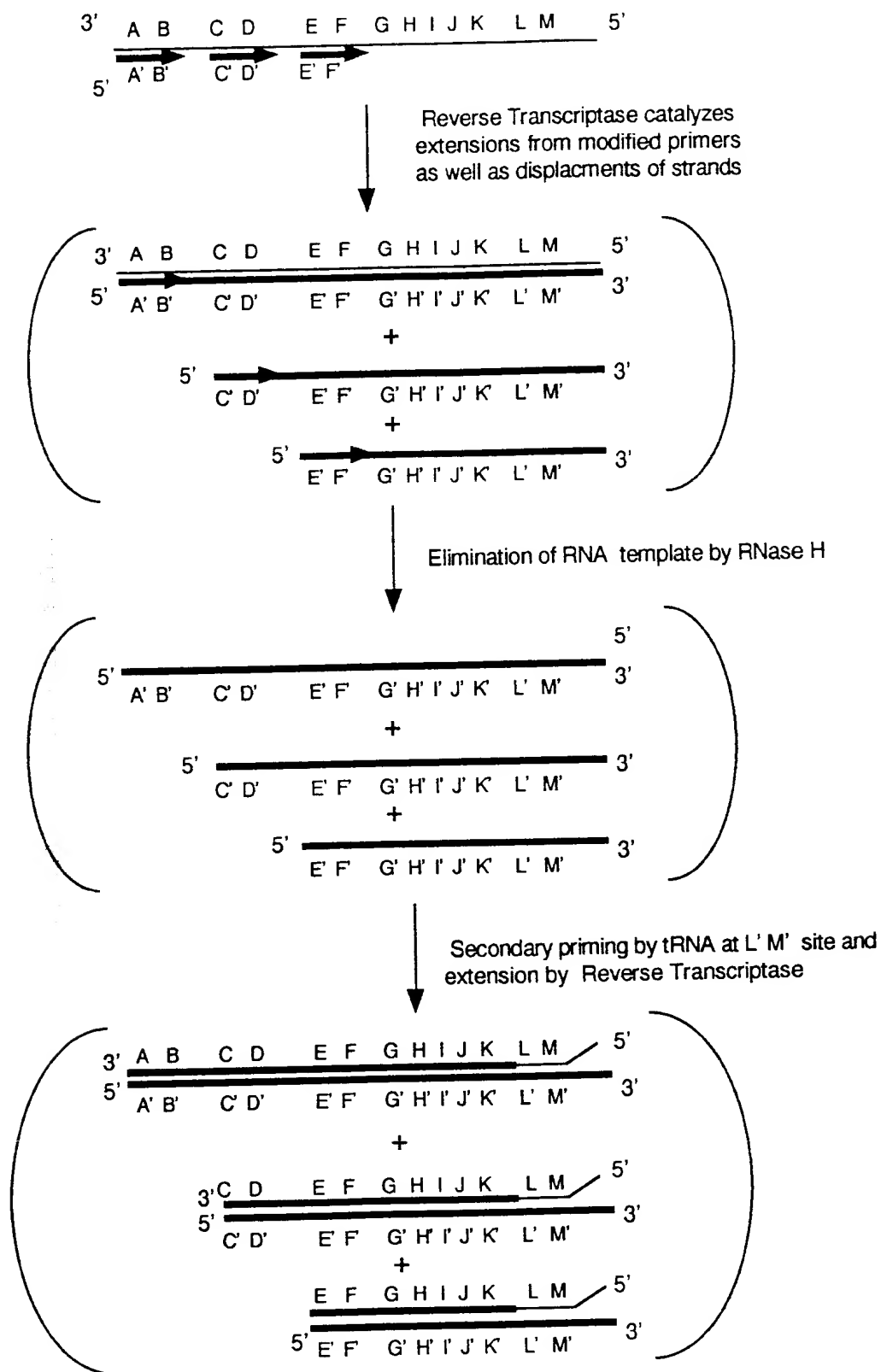
— = DNA
 — = RNA



(Continued in Figure 10)

Figure 9
 RNA with Ligands on Multiple Primers

Continued from Figure 9

**Figure 10**

RNA with Ligands on Multiple Primers (Continued)

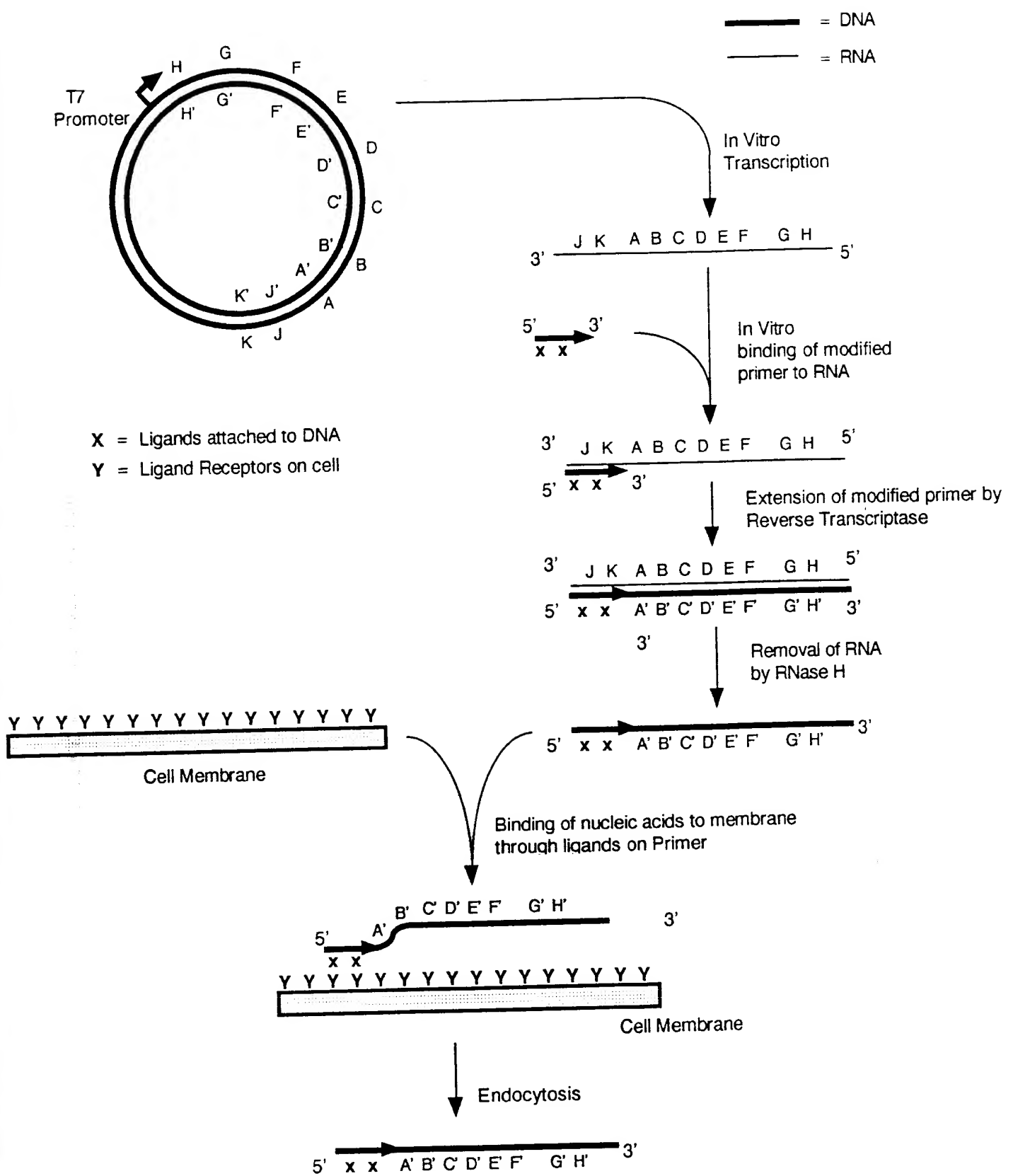
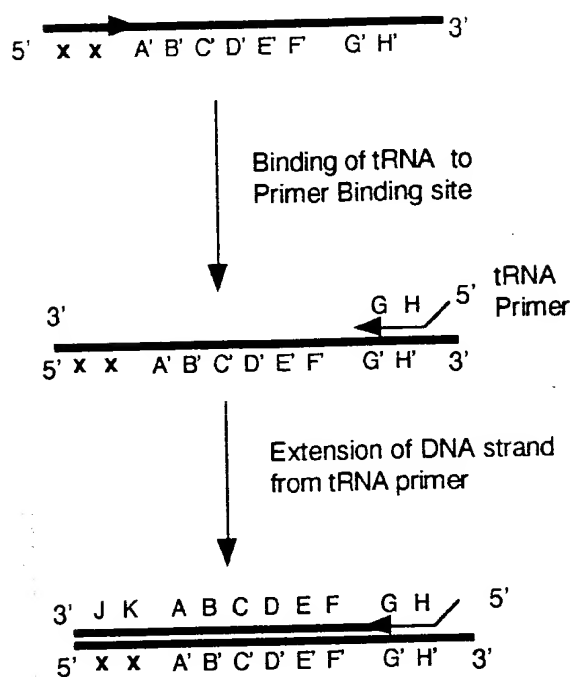


Figure 11
 Single-stranded DNA with attached Ligands

Continued from Figure 11

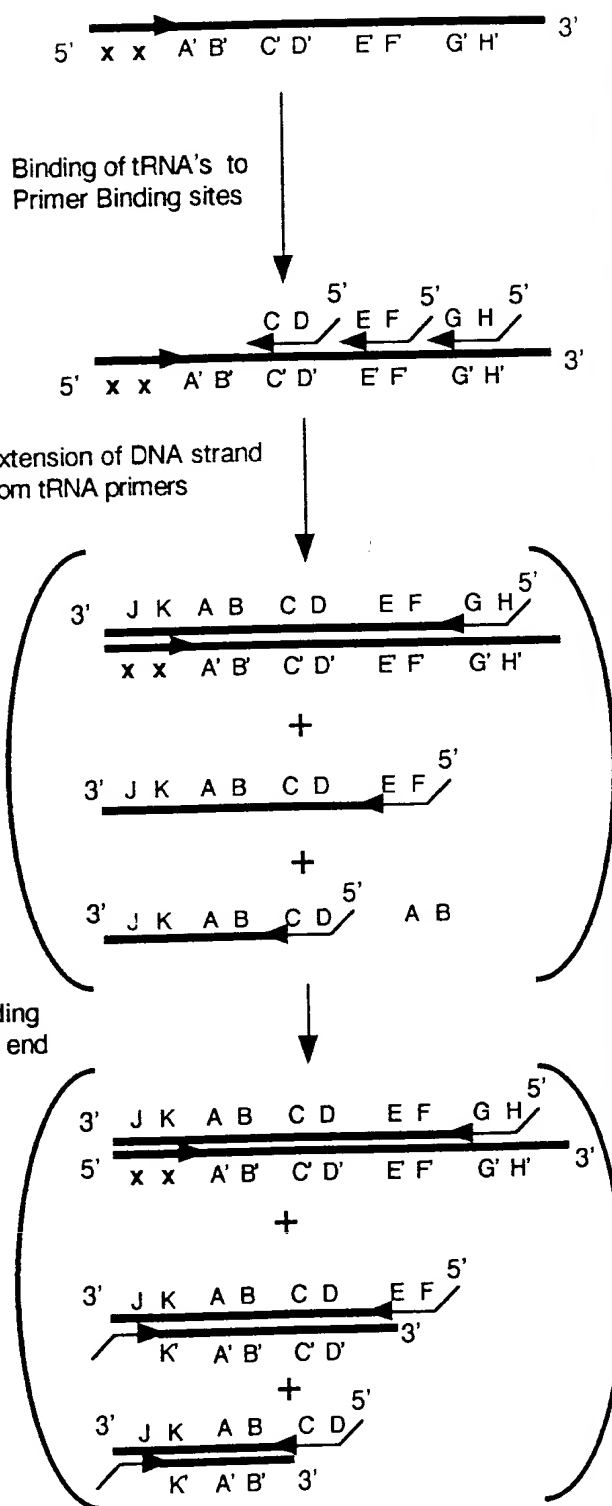
(a)

Presence of a single
tRNA primer site



(b)

Presence of multiple
tRNA primer sites



Synthesis of second strand by binding
of tRNA to Primer Binding site at 5' end

Figure 12

Single-stranded DNA with attached Ligands (continued)

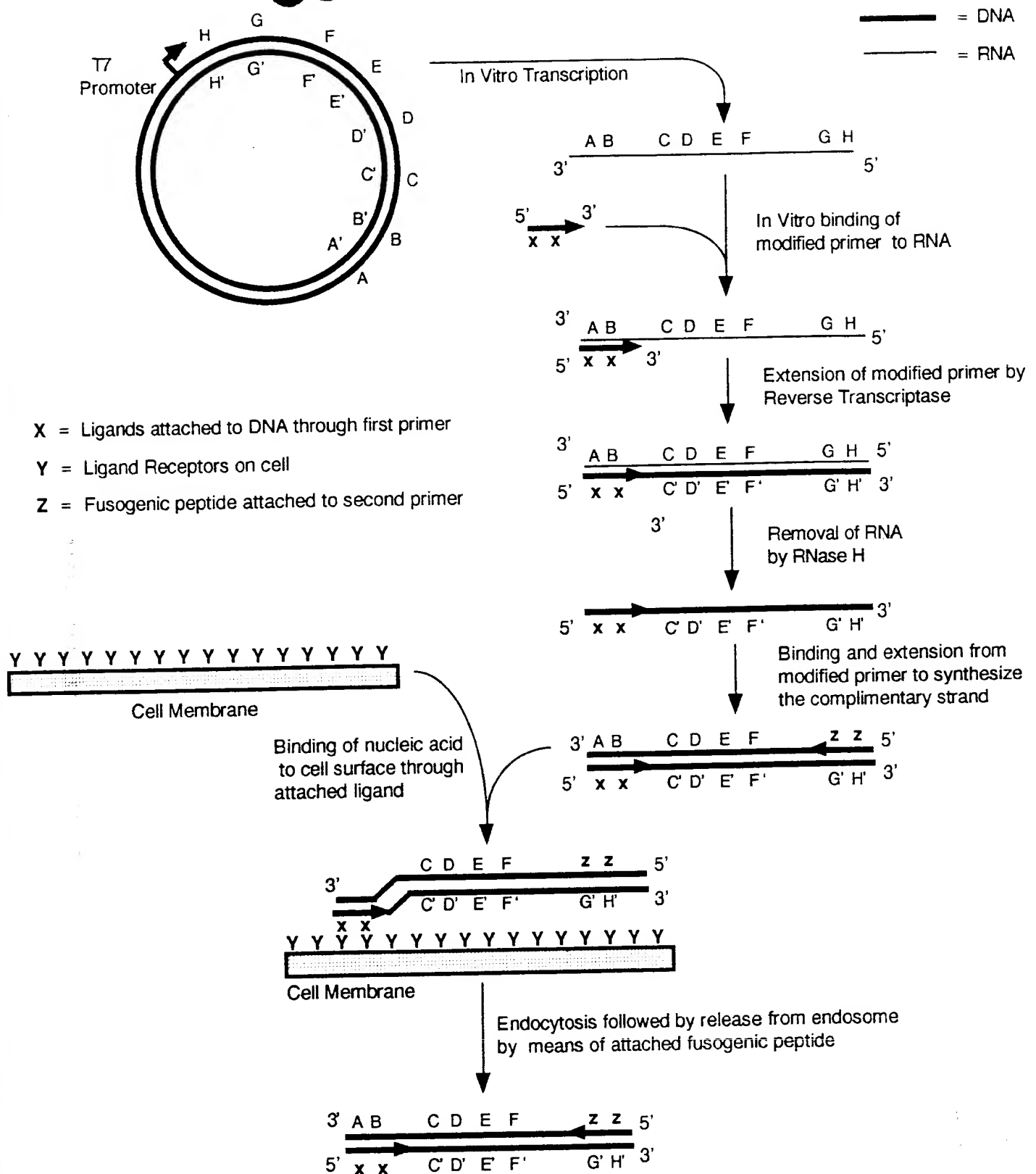


Figure 13

Linear Double-stranded DNA with attached Moieties on each strand

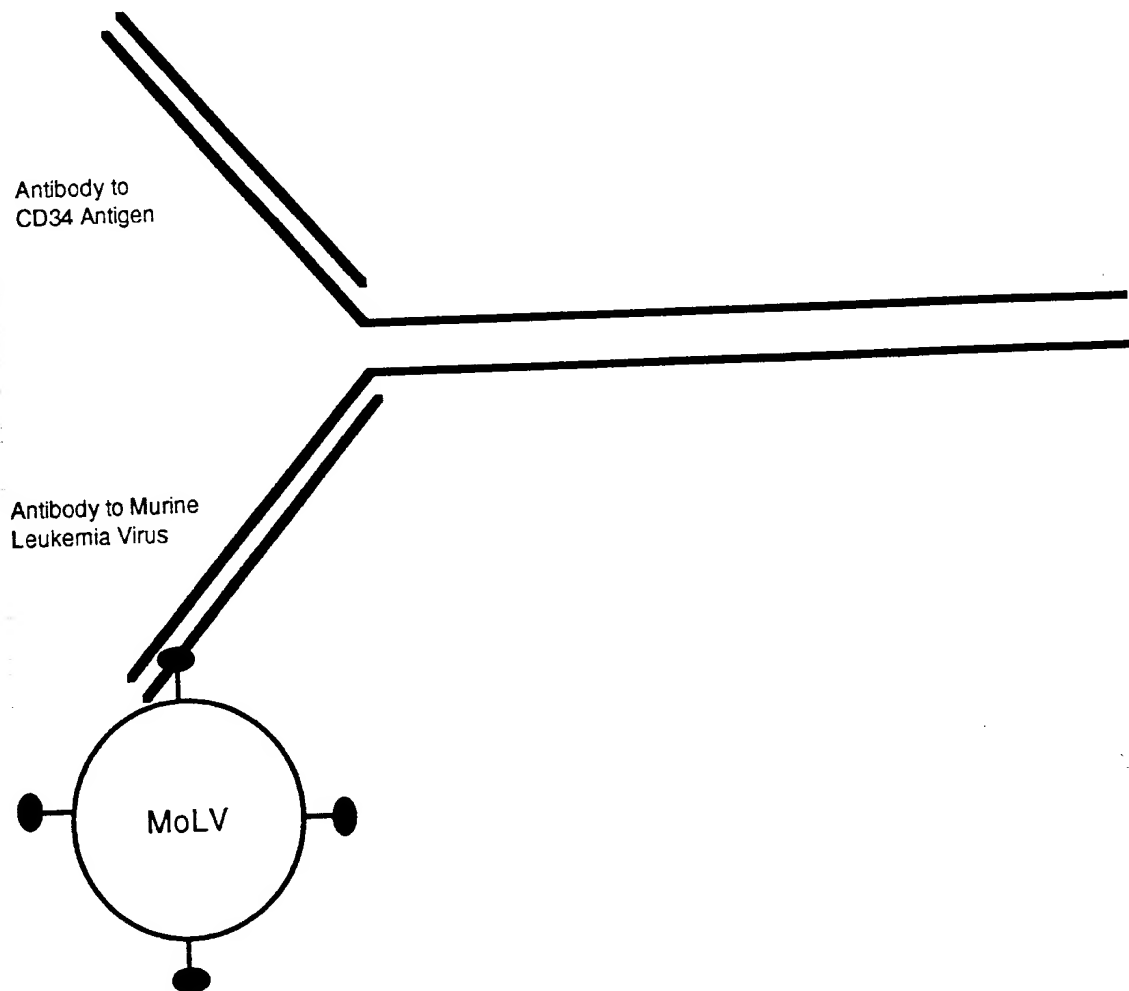


Figure 14

Enhanced Delivery of Retroviral Vector
to Haematopoietic Stem Cell

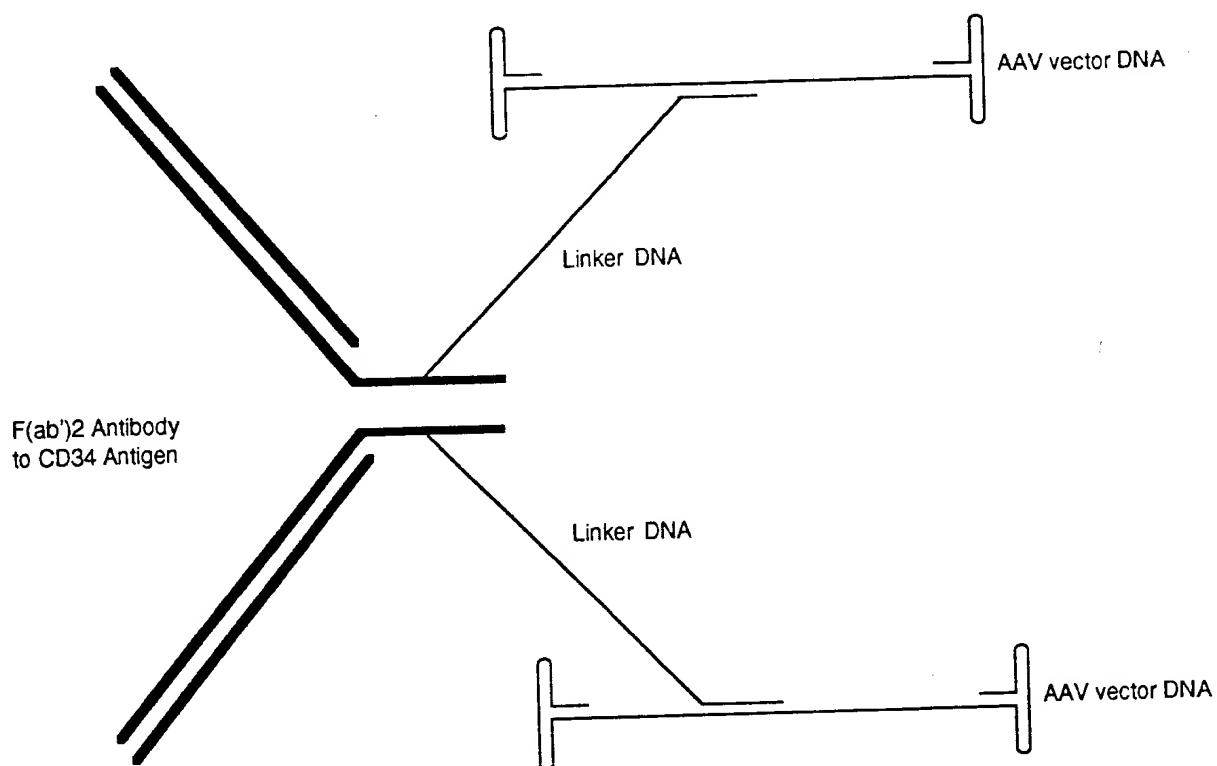


Figure 15

Enhanced Delivery of Vector
DNA to Haematopoietic Stem Cell

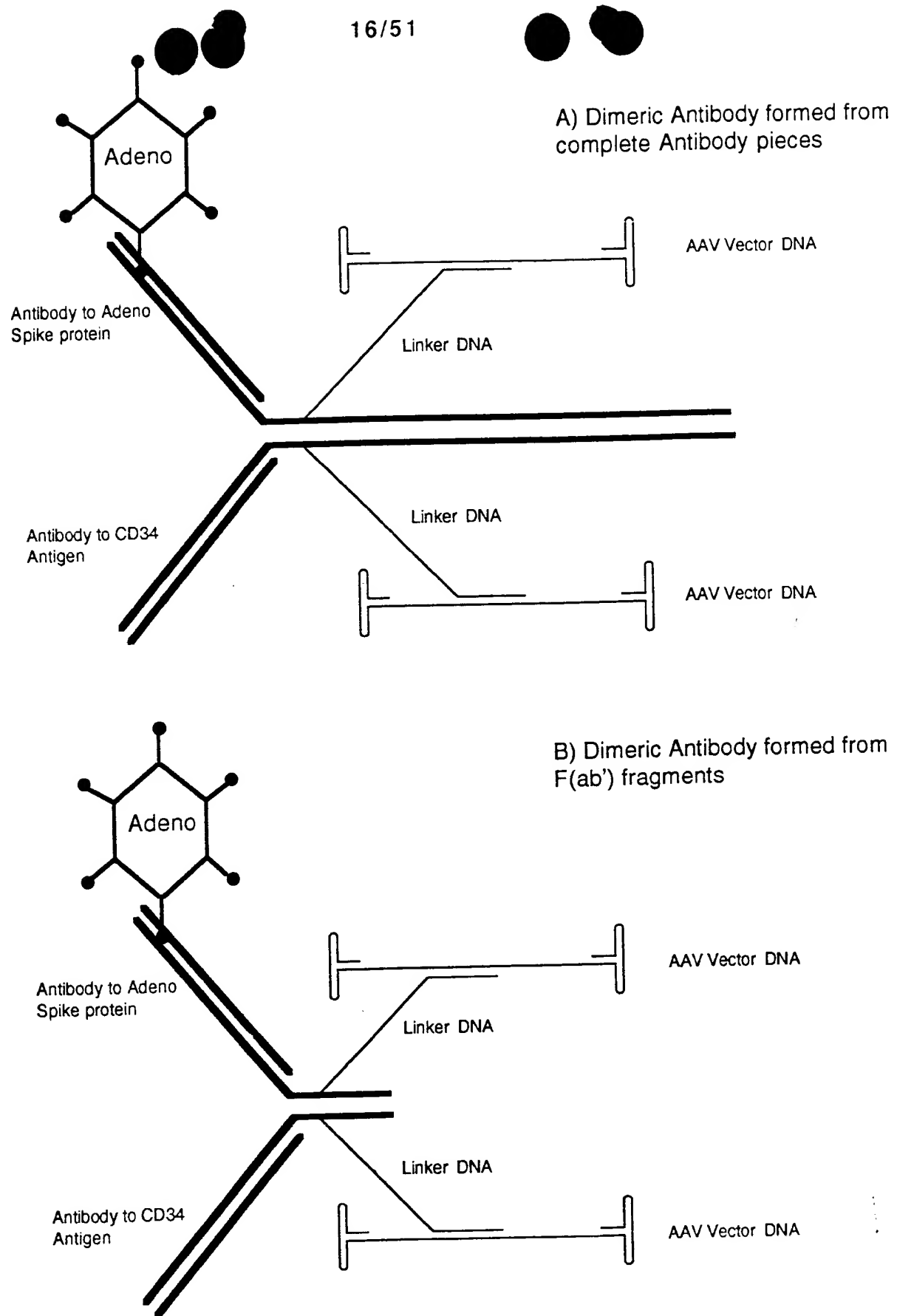


Figure 16
Covalent Attachment of vector DNA to Dimeric Antibody

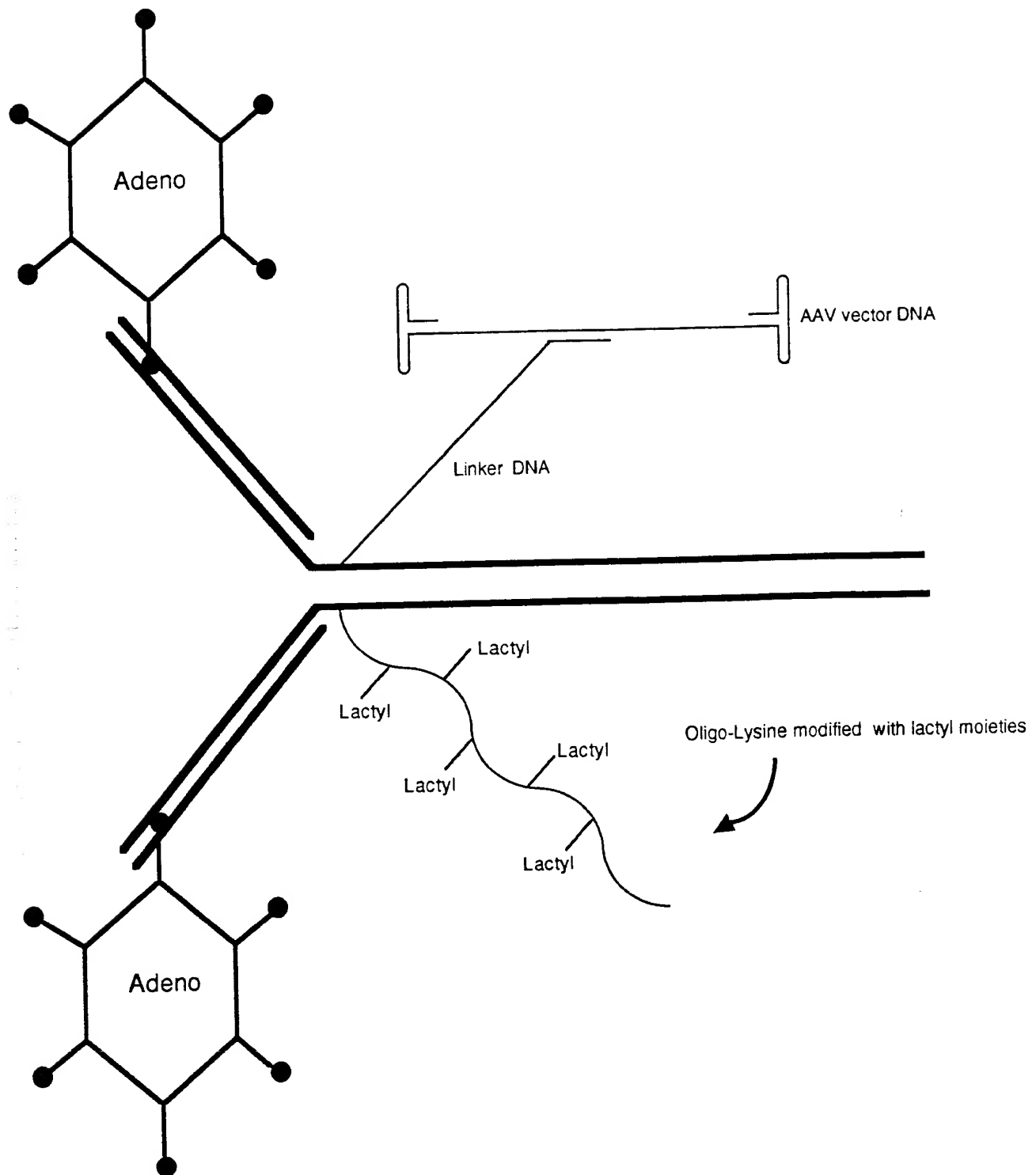


Figure 17
Covalent attachment of Modified DNA
to a Monovalent Antibody

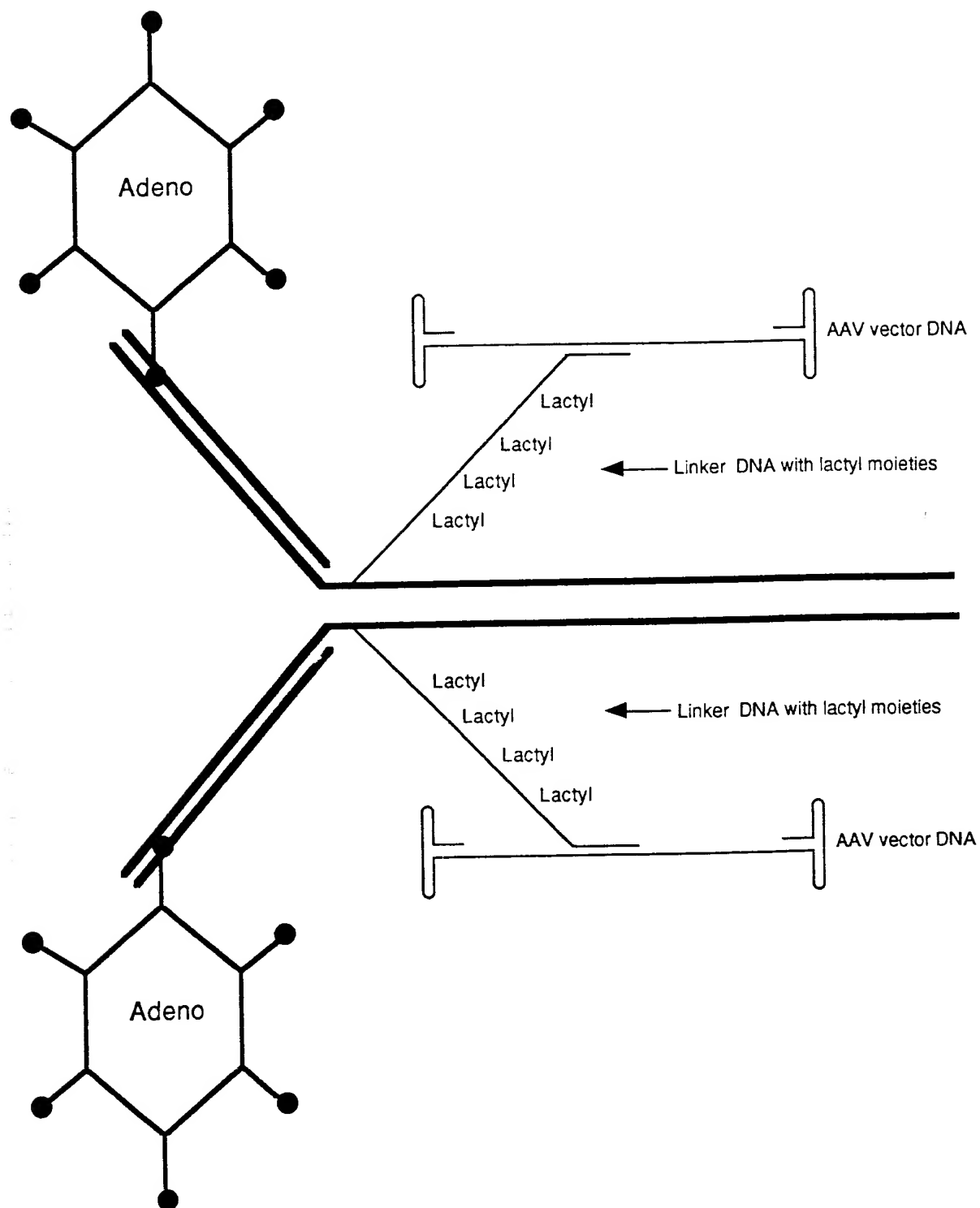
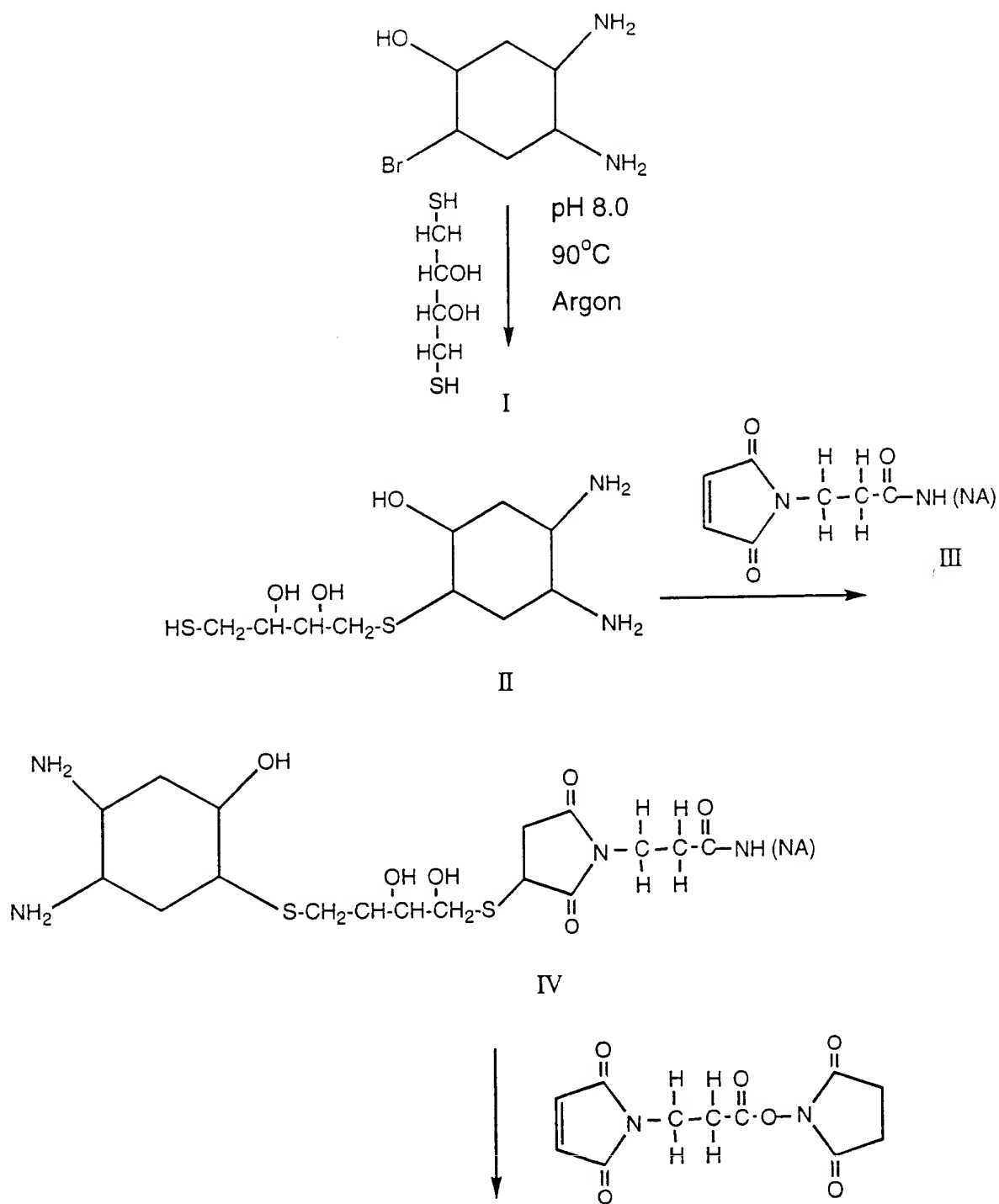


Figure 18
Modified DNA used as a Binder



(continued in Figure 20)

Figure 19
Synthetic Steps for Creation of Antibodies
With Nucleic Acid Moieties Attached

(Continued from Figure 19)

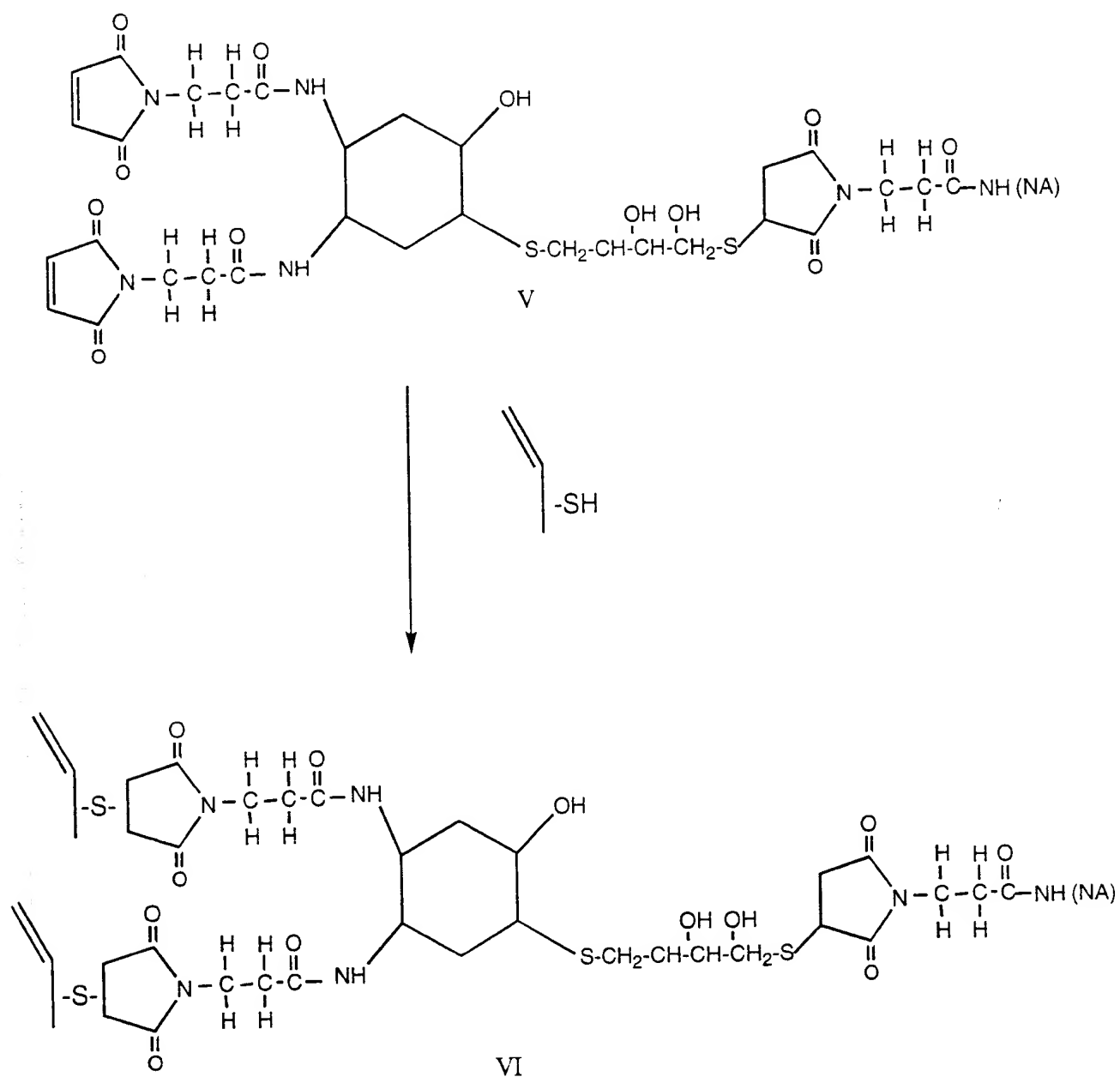


Figure 20
Continuation of Synthetic Steps

F(ab')₂ Antibody
to HIV p24
Antigen

F(ab')₂ Antibody
with Nucleic Acid
homopolymer

Multimerization of
F(ab')₂ Antibodies
by hybridization of
Nucleic Acid
homopolymers

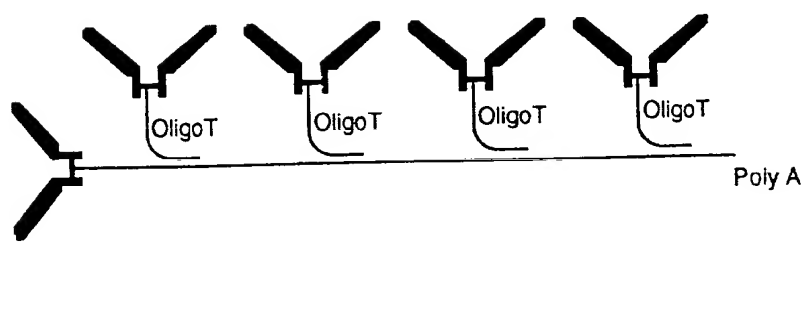


Figure 21

Enhanced Binding of Antibodies to Antigens by Multimerization

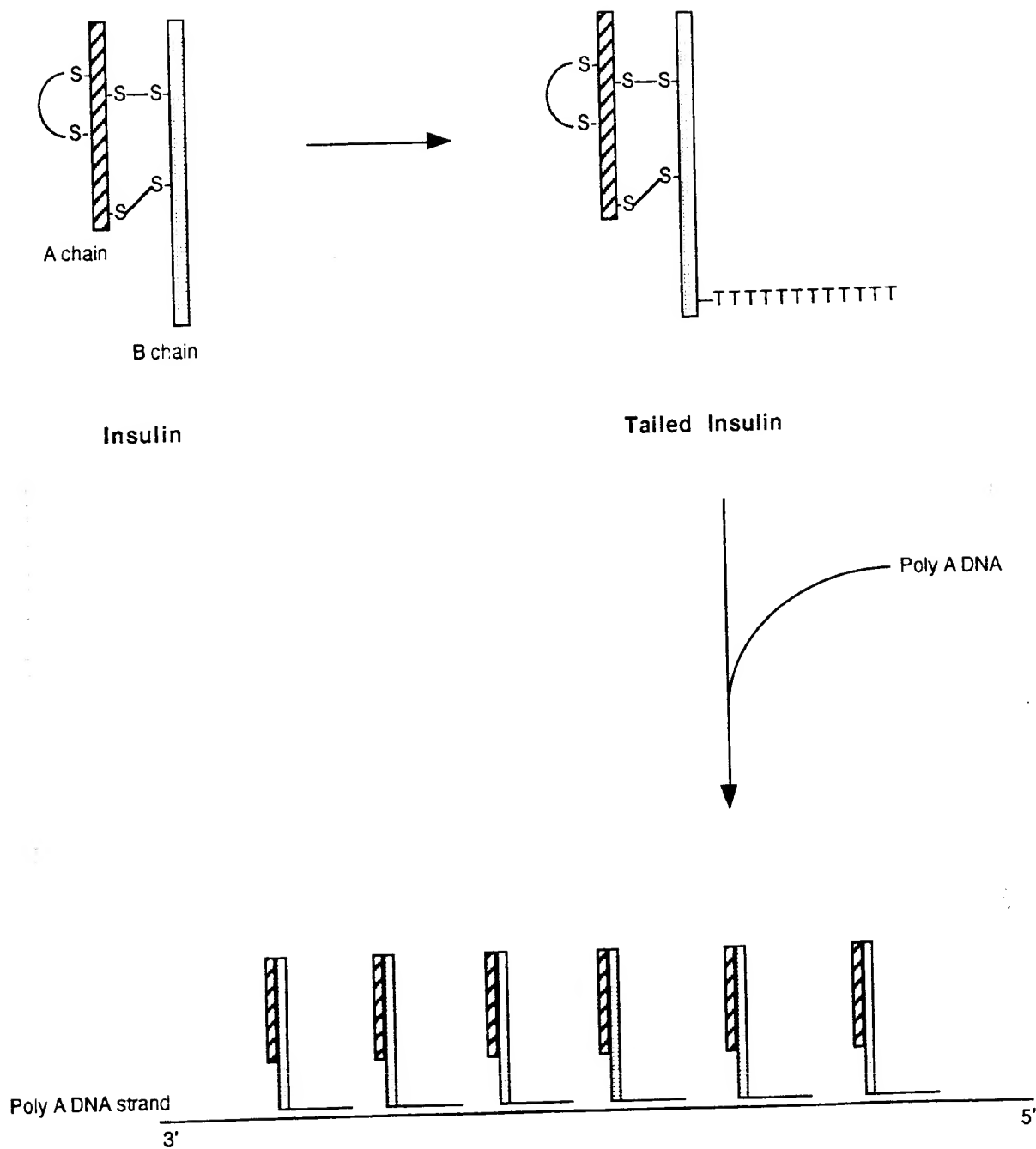


Figure 22
High Affinity Multi-Insulin Soluble Complex

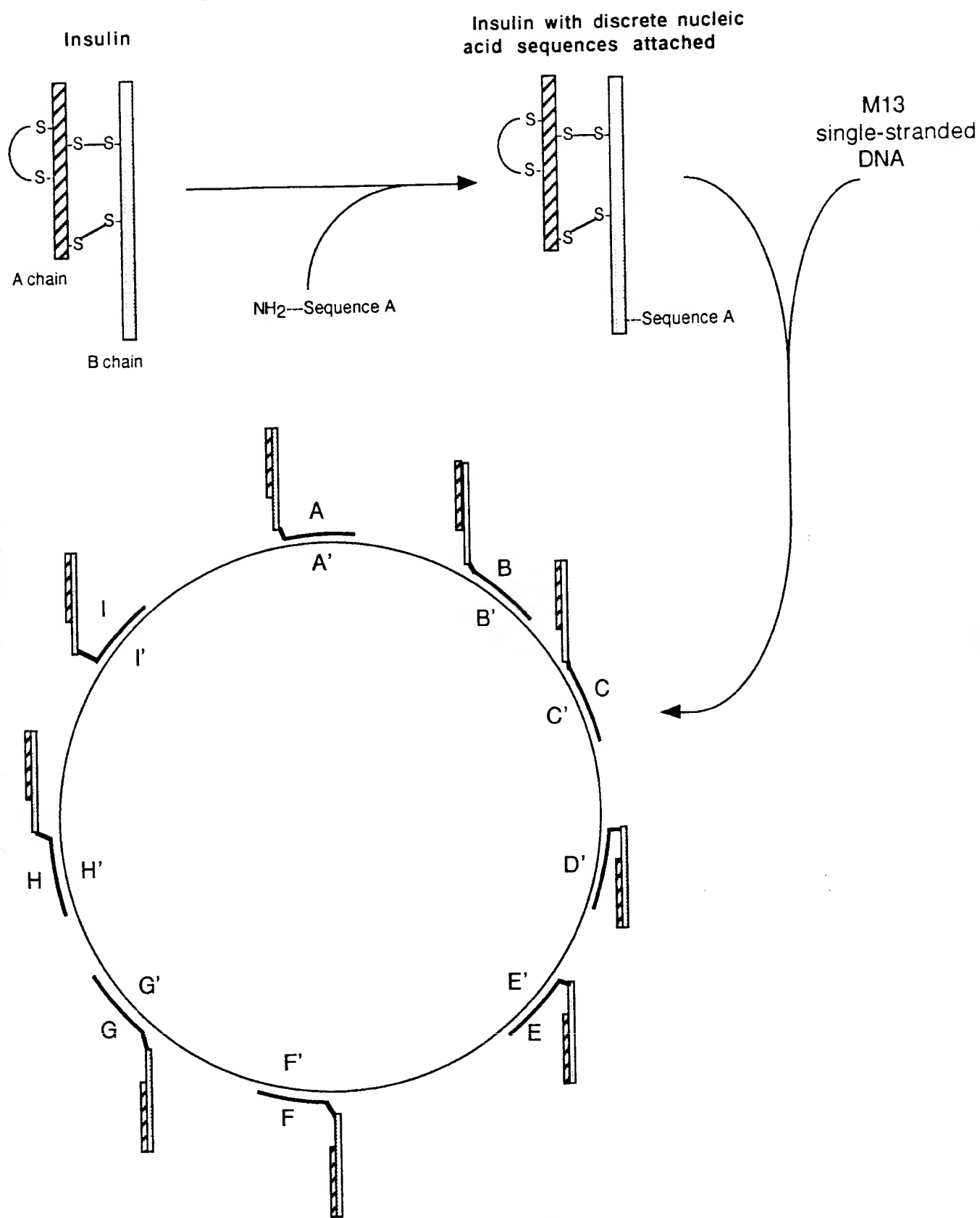


Figure 23

Multimerization of Insulin molecules by hybridization to discrete Sequences

T7 RNA Polymerase Sequence

↓

↓

SV40 Intron Sequence

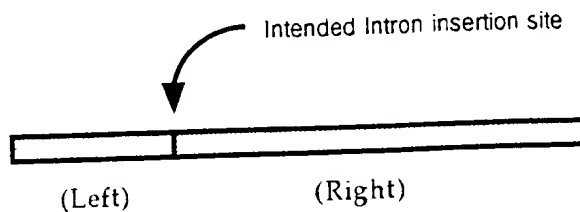
Insertion of SV40 Intron into polymerase coding sequence

mRNA transcript containing intron

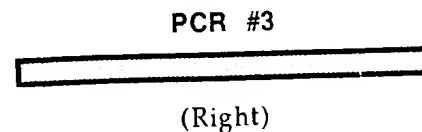
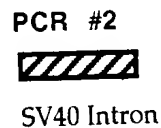
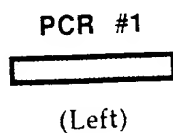
mRNA transcript after splicing has normal T7 Sequence

Fusion of Intron into T7 RNA Polymerase Coding Sequence

Normal T7 RNA polymerase
coding sequence

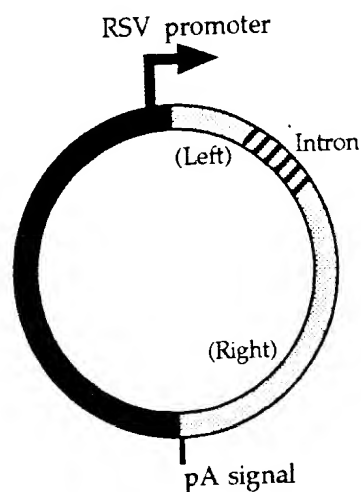


Synthesis of fragments by
PCR Amplification of T7 or
SV40 templates



(A)

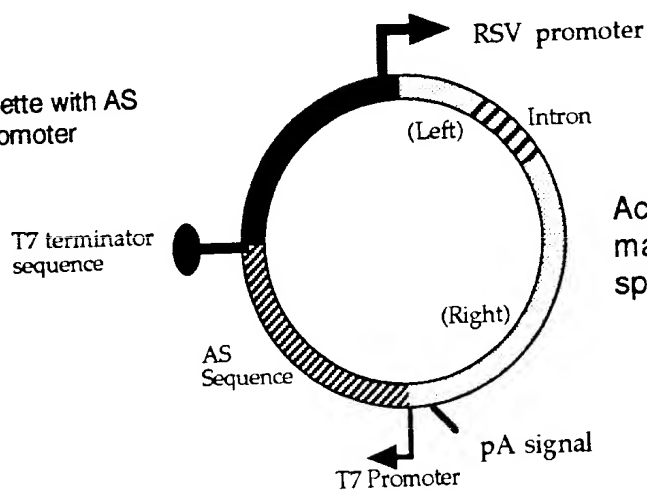
Fusion of PCR fragments
together in eucaryotic
expression vector



(B)

Introduction of cassette with AS
directed from T7 promoter

(C)

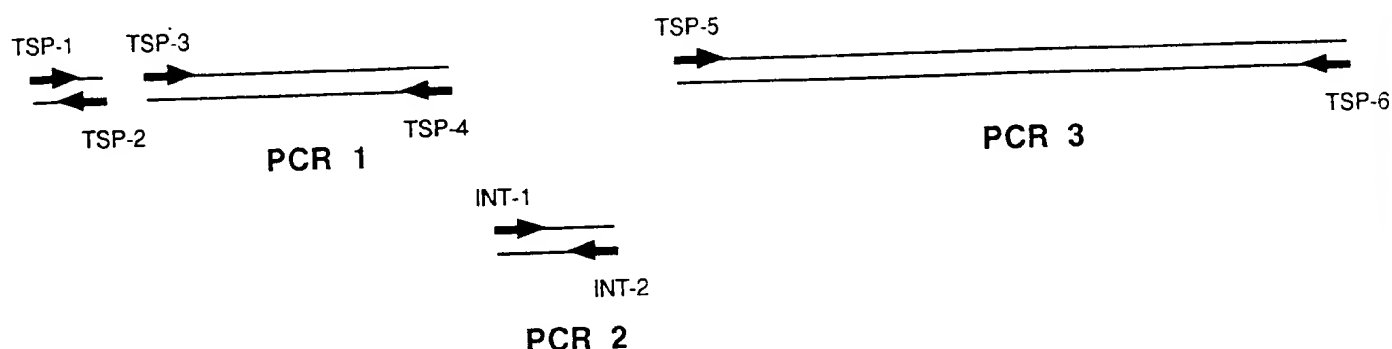


Active T7 RNA polymerase is only
made in eucaryotic cells after
splicing out of SV40 Intron

Figure 25

Construction of T7 Expression Vector

A) Synthesis of pieces



B) Oligomers used for synthesis

TSP-1	GGA ATT CGT CTC GAG CTC TGA TCA CCA CCA TGG ACA CGA TTA ACA TCG C
TSP-2	GAC TAG TTG GTC TCG TCT CTT TTT TGG AGG AGT GTC GTT CTT AGC GAT GTT AAT C
TSP-3	GGA ATT CGT CTC GGA GAA AGG TAA AAT TCT CTG ACA TCG AAC TGG C
TSP-4	GAC TAG TGG TCT CCC CTT AGA GAG CAT GTC AGC
TSP-5	GGA ATT CGG TCT CGG GTC TAC TCG GTG GCG AGG
TSP-6	GAC TAG TCG TTA CGC GAA CGC AAA GTC
INT-1	GGA ATT CGT CTC TAA GGT AAA TAT AAA ATT TTT AAG
INT-2	GAC TAG TCG TCT CTG ACC CTA AAA TAC ACA AAC AAT TAG A

Figure 26

Synthesis of Pieces for Construction of
T7 RNA Polymerase with Intron

Formation of Nuclear Localisation Signal by Fusion of TSP1/TSP2 Product to Clone with PCR #1 product

Annealing of TSP1 with TSP2

TSP1

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GC 3' TSP2
3' C TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GCT CTG GTT GAT CAG 5'

Extension of TSP1/TSP2 by polymerase

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA CGA GAC CAA CTA GTC 3'
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GAT CAG 5'
Bsa I

Digestion of TSP1/TSP2 product with Bsa I

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AA
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT

Digestion of PCR #1 clone (pL-1) with BsmB I

Bsm B I
5' GGA ATT CGT CTC G GAGA AAG GTA AAA TTC TCT GAC ATC GAA CTG GC-----
CCT TAA GCA GAG CCTCT TTC CAT TTT AAG AGA CTG TAG CTT GAC CG-----

Ligation of Bsa I digested TS1/TS2 product to BsmB I digested PCR#1 clone

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA AAC GTA AAA TTC
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT TTC CAT TTT AAG

TCT GAC ATC GAA CTG GC-----
AGA CTG TAG CTT GAC CG-----

Figure 27

Comparison of the 5' ends of the Nucleotide Sequences of Wild Type and Modified T7 RNA Polymerase

Wild Type T7 nucleic and amino acid sequence

ATG	GAC	ACG	ATT	AAC	ATC	GCT	AAG	AAC	GAC	TTC	TCT	GAC	ATC	GAA	CTG	GC-----
TAC	CTG	TGC	TAA	TTG	TAG	CGA	TTT	CTG	AAG	AGA	CTG	TAG	CTT	GAC	CG-----	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	

Modified T7 nucleic and amino acid sequence with Nuclear Localisation Signal (NLS) insertion

ATG	GAC	ACG	ATT	AAC	ATC	GCT	AAG	AAC	GAC	ACT	CCT	CCA	AAA	AAG	AGA	AGA	GTA	AAA	TTC	TCT	GAC	ATC	GAA	CTG	GC-----
TAC	CTG	TGC	TAA	TTG	TAG	CGA	TTT	CTG	AAG	AGA	CTG	TAG	CTT	GAC	CG-----										
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16										

Figure 28

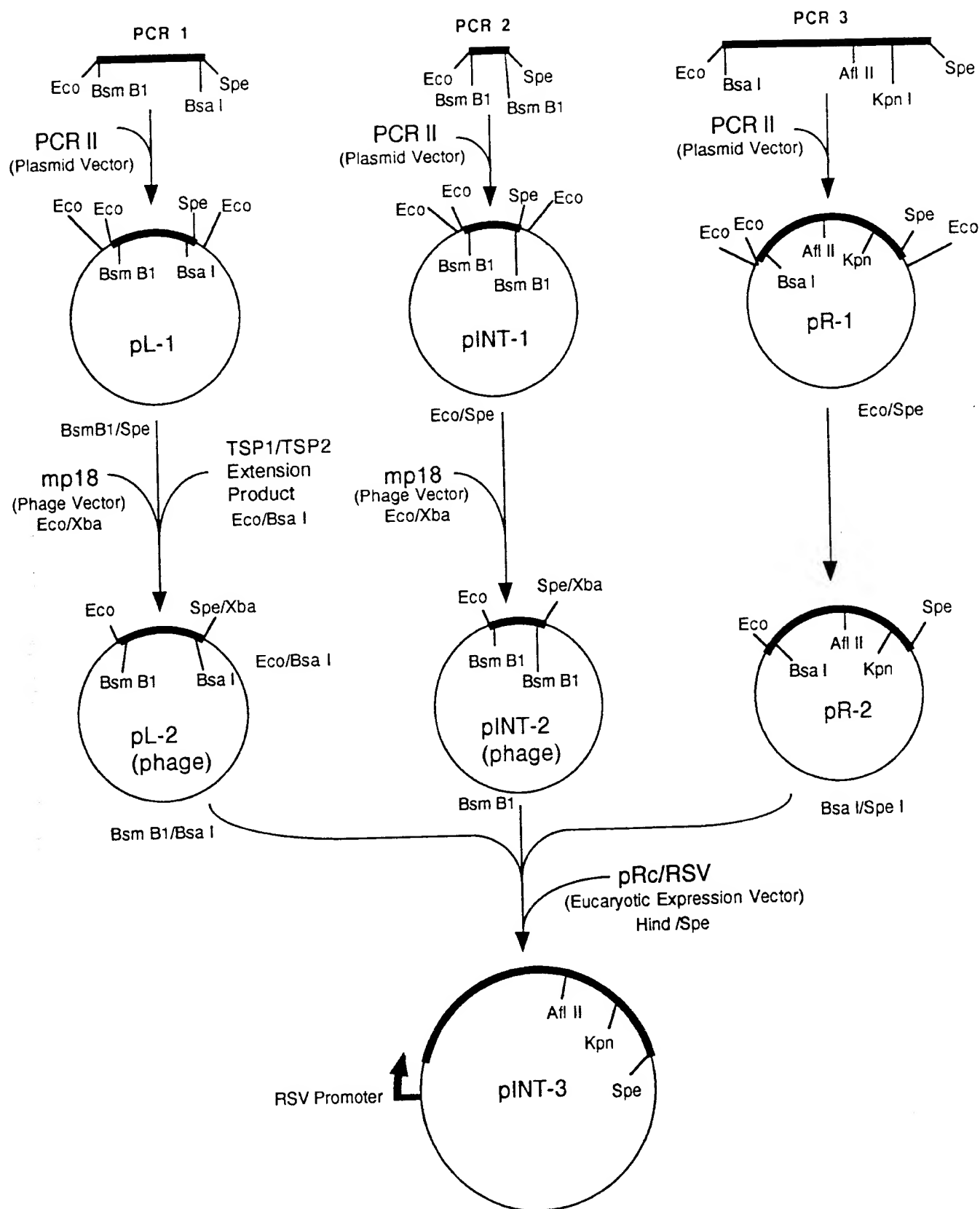


Figure 29

Fusion of PCR Pieces to Construct
T7 RNA Polymerase with an Intron

(A) Oligomers

HTA-1	GAT CAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGCTTA AGC CTC AAG
HTA-2	GAT CCT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT
HTB-1	GAT CAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA G
HTB-2	GAT CCT TGA GGA GGT CTT CGT CGC TGT CTC CGC TTC TTC CTG CCA TAG GAG AGC CTA AGG T
HTC-1	GAT CAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC AG
HTC-2	GAT CCT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT
TER-1	AAT CTA GAG CTA ACA AAG CCC GAA AGG AAG
TER-2	TTC TGC AGA TAT AGT TCC TCC TTT CAG C

(B) Cloning of AS and Terminator sequences into vector with T7 Promoter

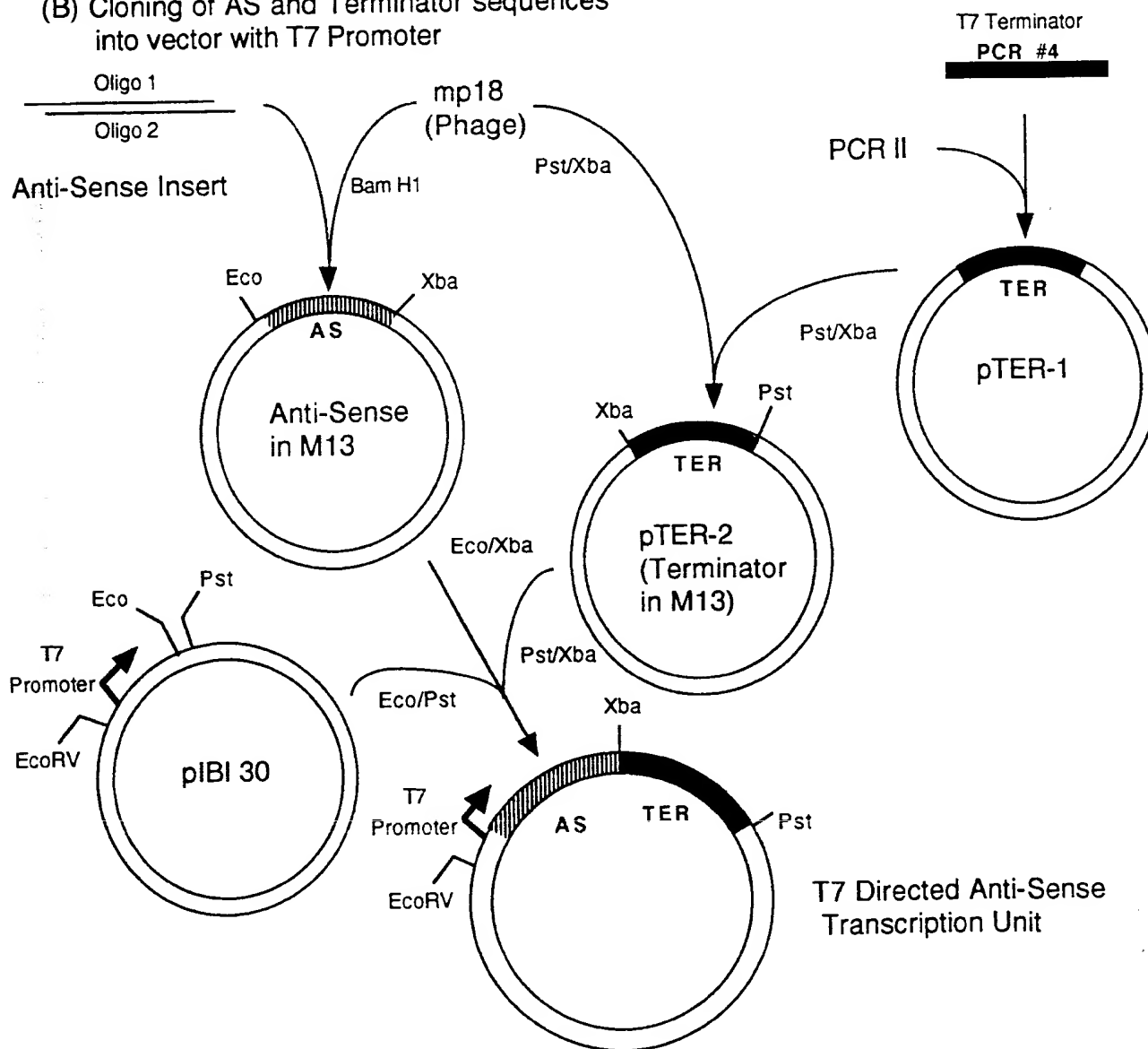


Figure 30
Insertion of Anti-Sense Sequences into
T7 Directed Transcription Units

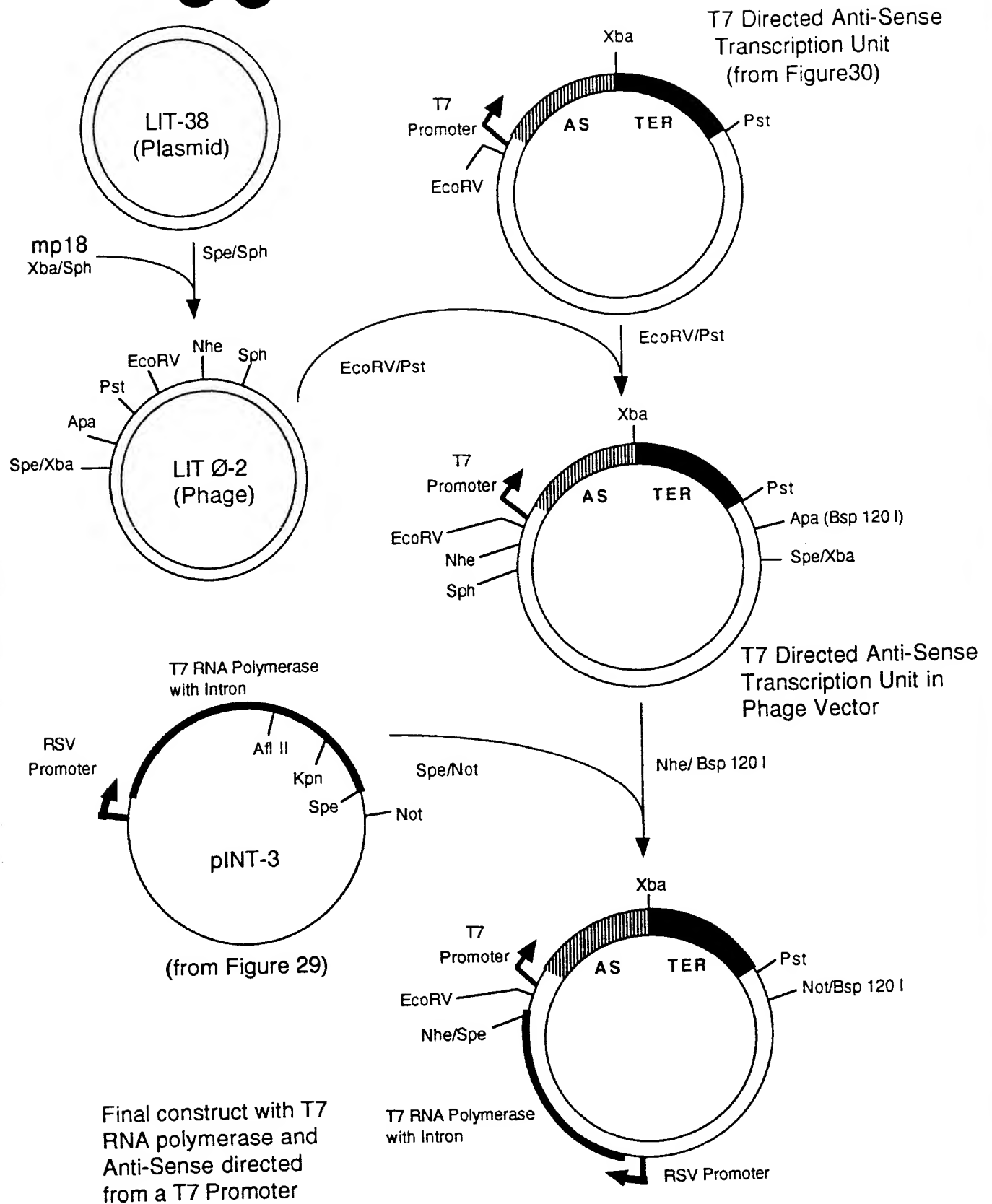


Figure 31

Construct with T7 RNA polymerase and Anti-Sense directed from a T7 Promoter

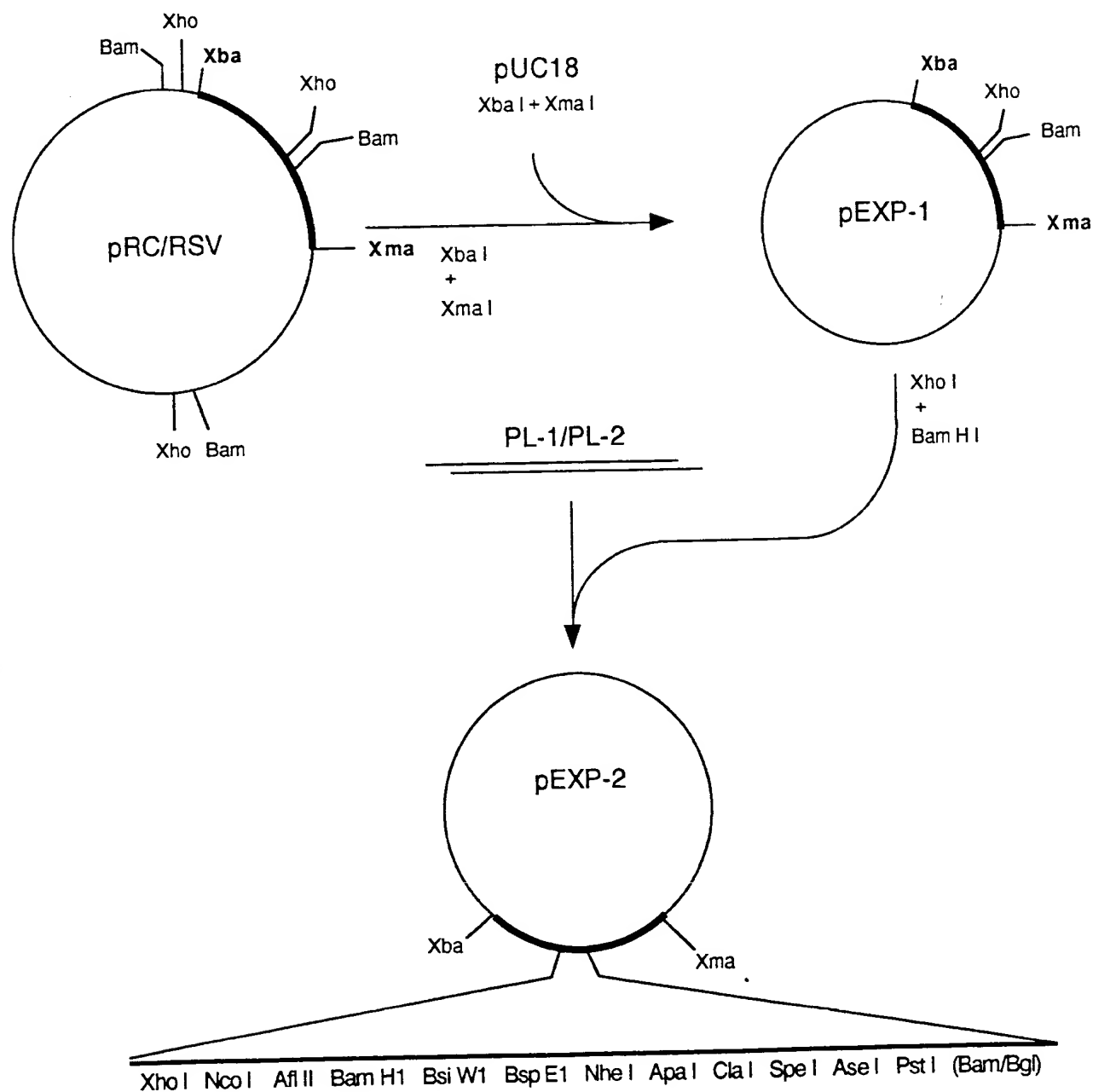
A) Oligomers for introduction of T7 signals and polylinker

PL-1

TCG AGC CAT GGC TTA AGG ATC CGT ACG TCC GGA GCT AGC GGG CCC ATC GAT ACT
AGT TAA ATG CAG ATC T

PL-2

CTA GAG ATC TGC ATT TAA CTA GTA TCG ATG GGC CCG CTA GCT CCG GAC GTA CGG
ATC CTT AAG CCA TGG C

**Figure 32**

Introduction of Poly-Linker for Creation of Protein Expression Vector

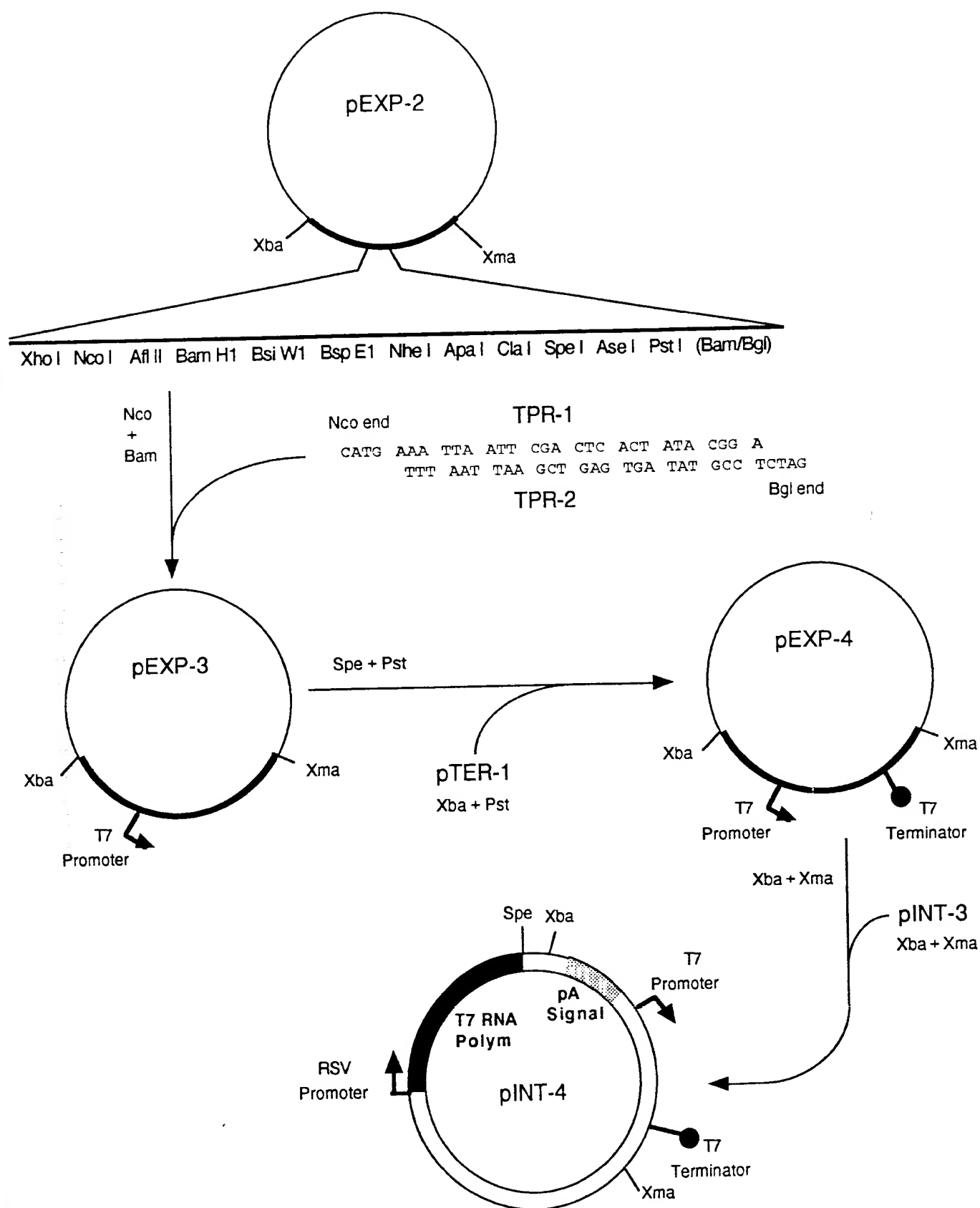
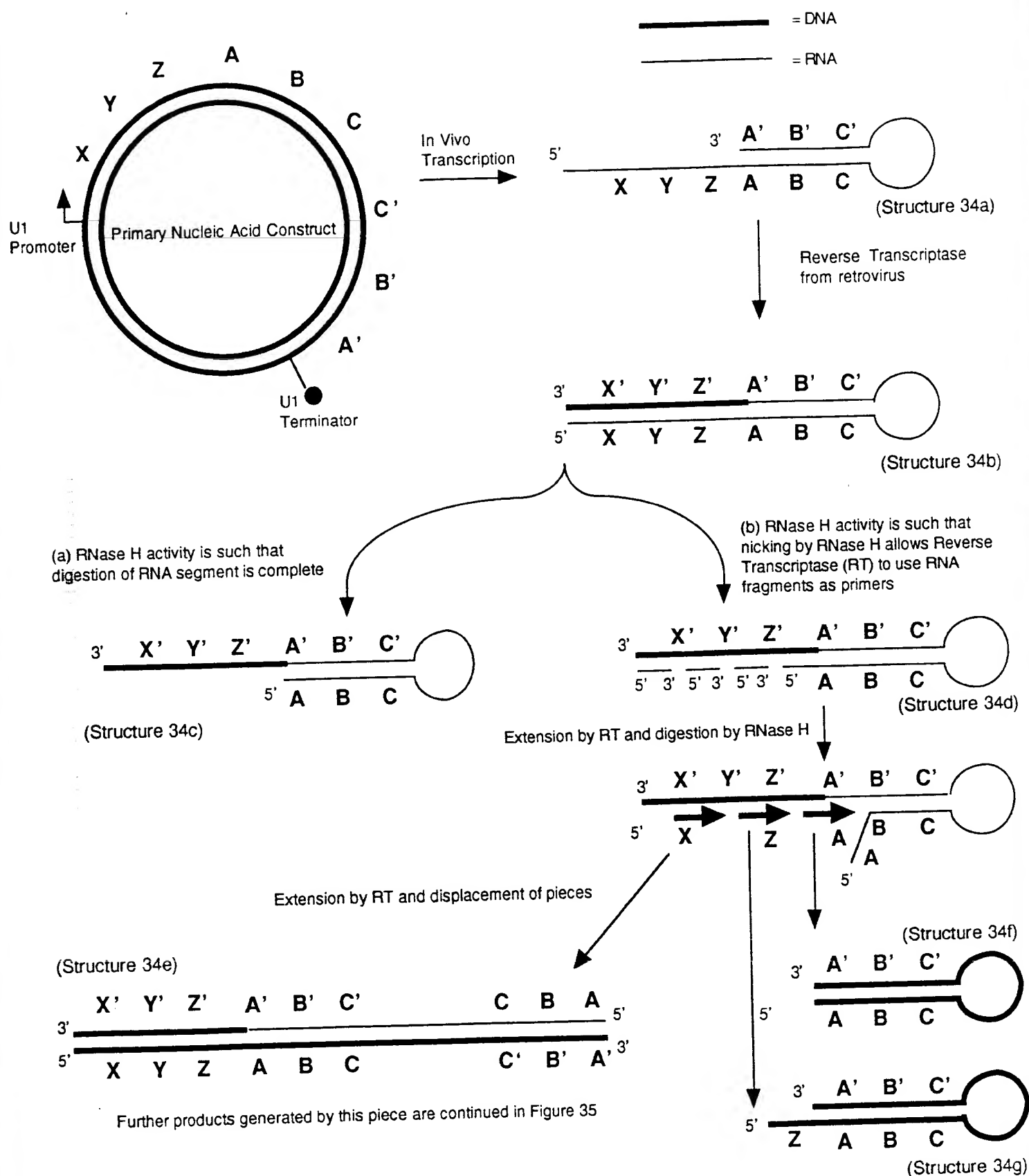


Figure 33

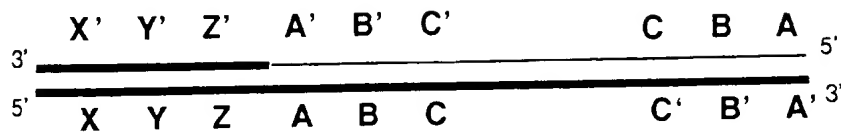
Final steps for construction of Expression Vector

**Figure 34**

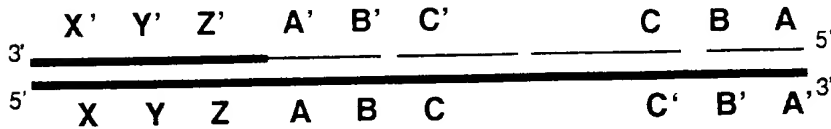
Construct that produces single-stranded Anti-Sense DNA

Continued from Figure 34

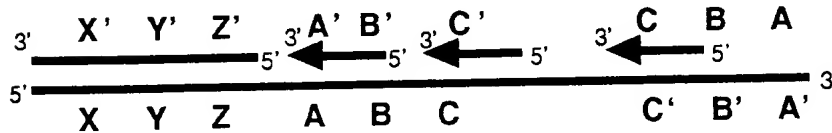
(Structure 34e)



Nicking by RNase H



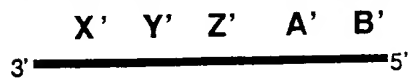
Extension by RT and digestion by RNase H



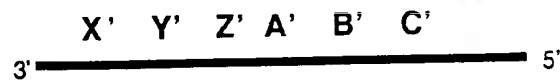
(Structure 35h)



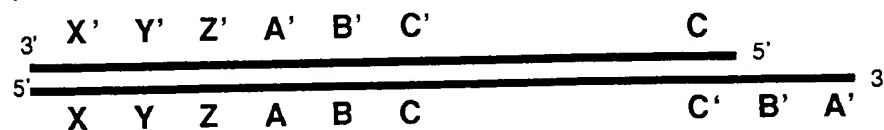
(Structure 35i)



(Structure 35j)



(Structure 35k)



Extension by RT and displacement generates Single-Stranded DNA and a mostly Double-stranded DNA molecule

Figure 35
Continuation of Process from Figure 34

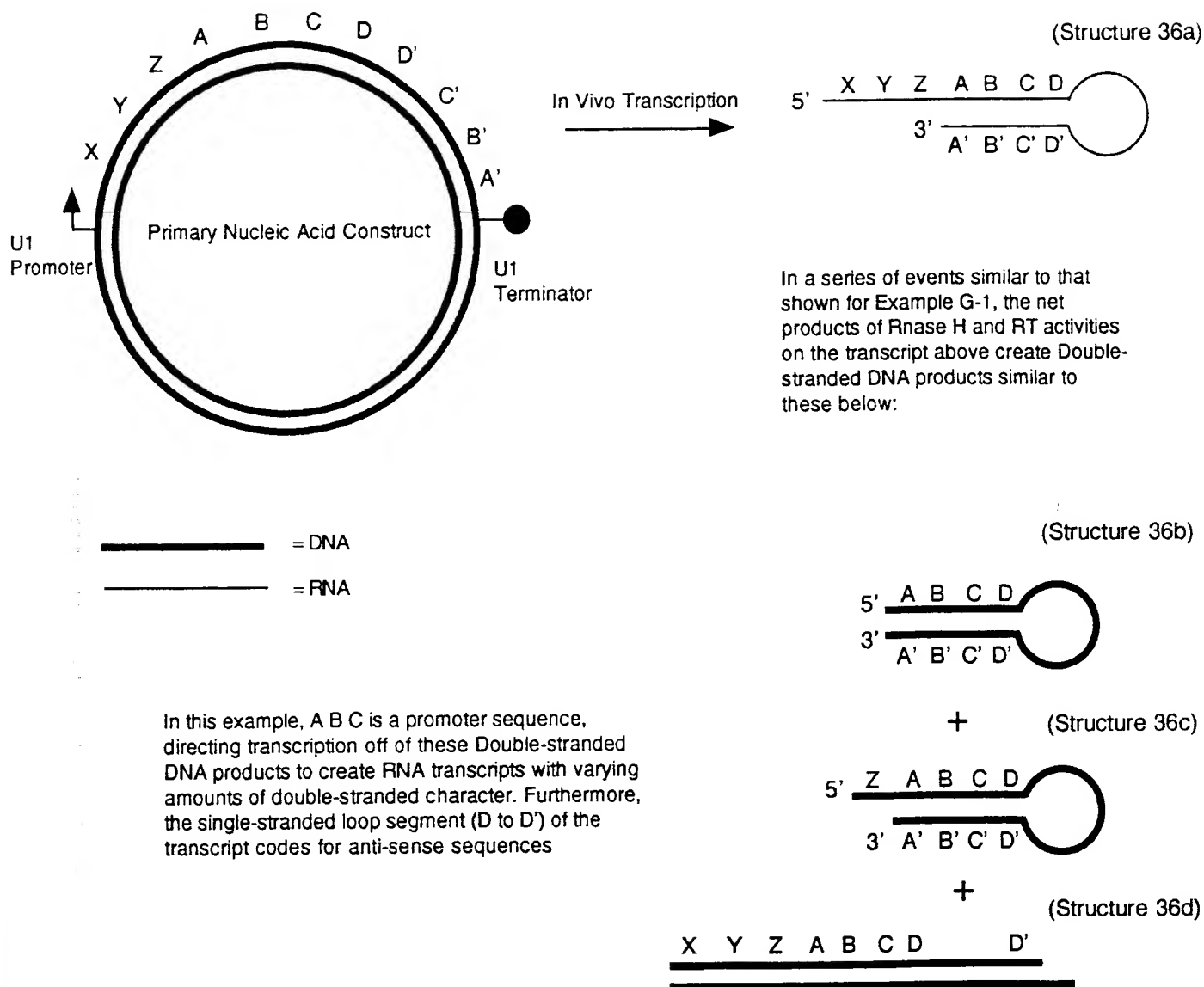
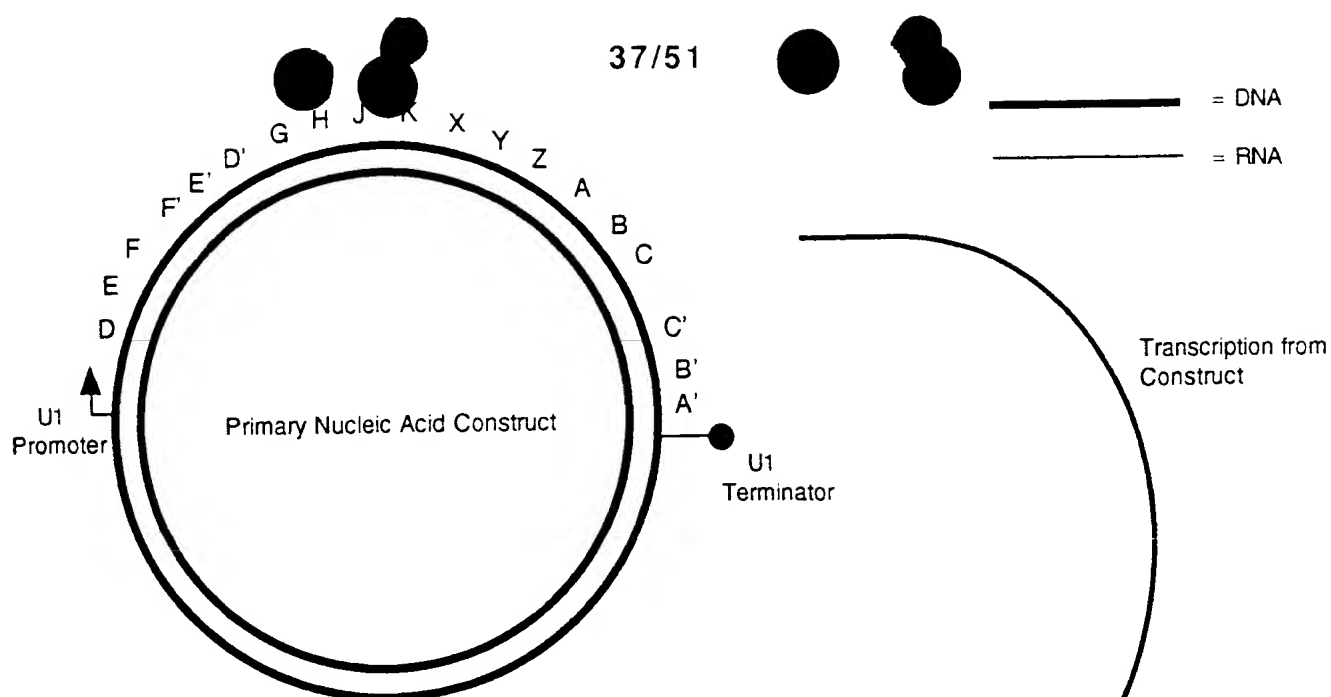
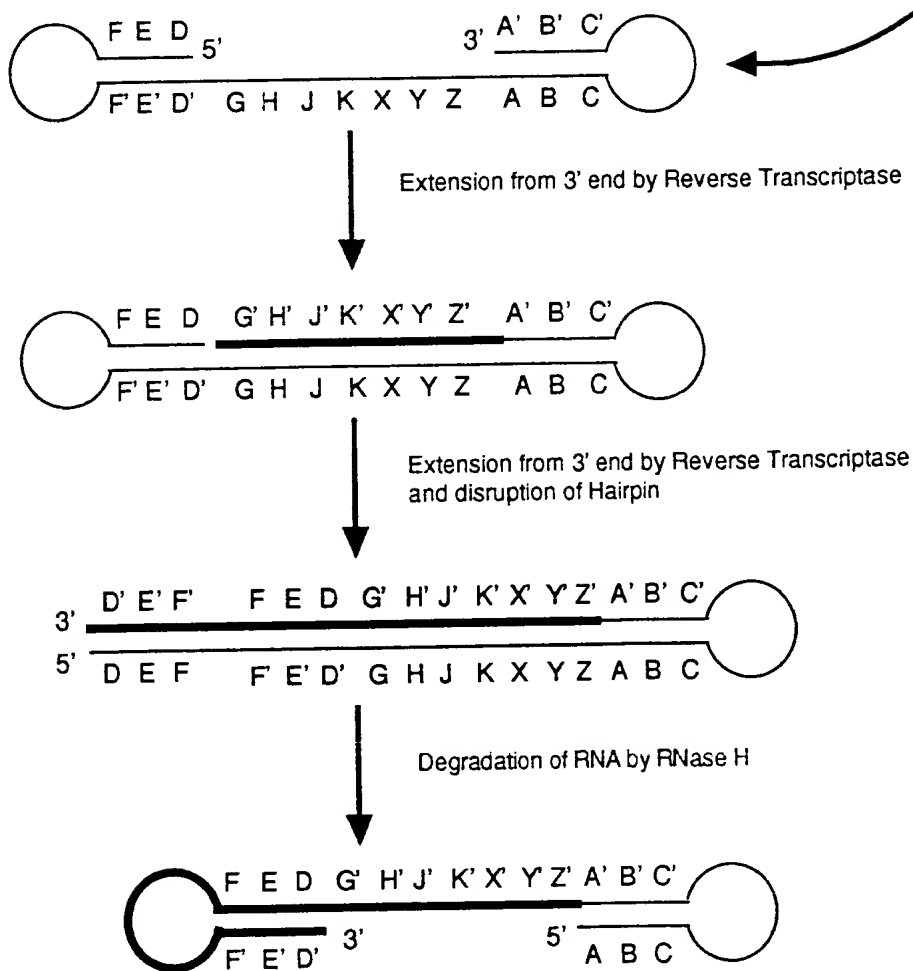


Figure 36

Construct that produces RNA that is Reverse Transcribed to create Secondary DNA Constructs capable of directing transcription

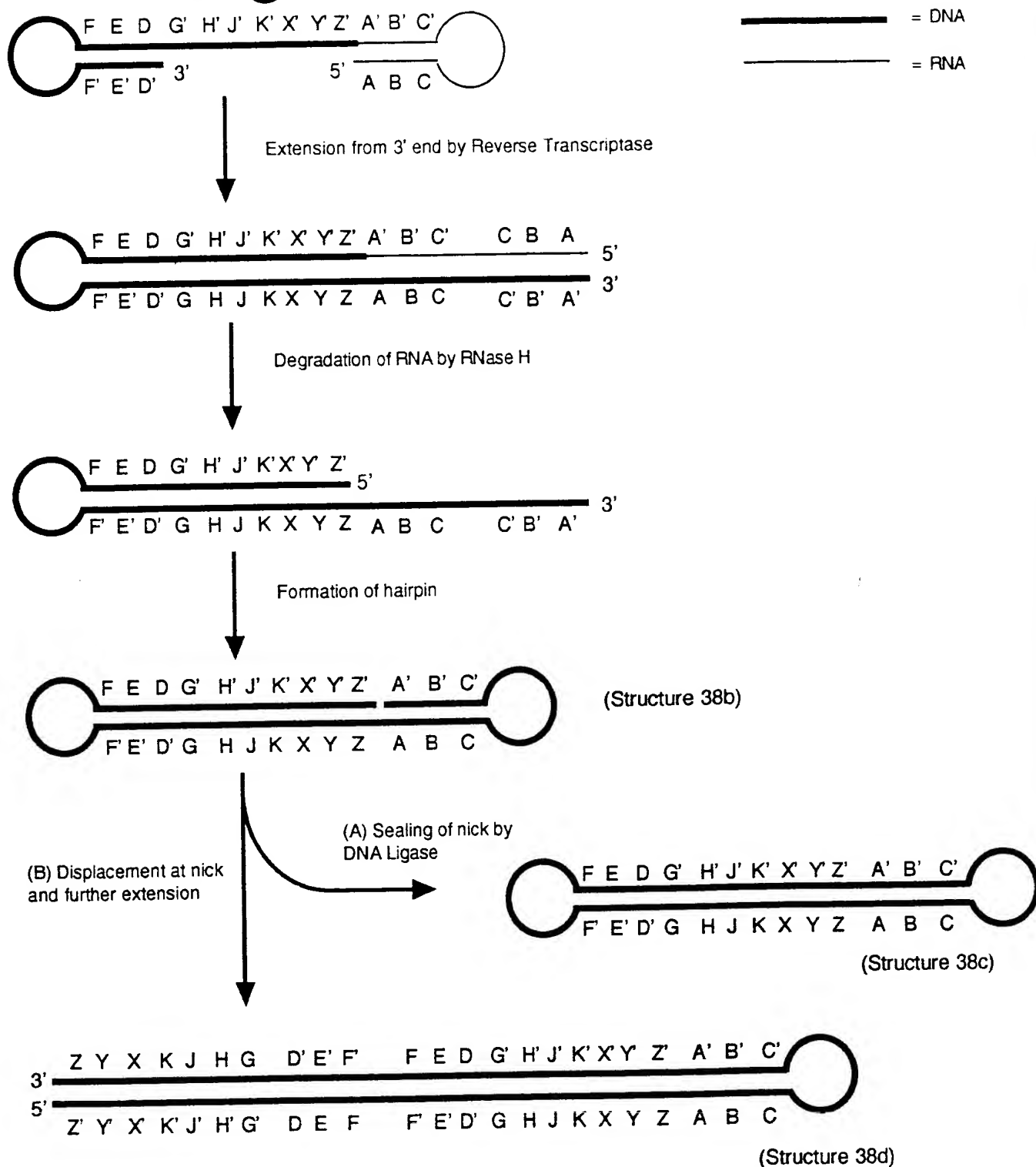


(Structure 37a)



(Continued in Figure 38)

Figure 37
Construct which Propagates a Double Hairpin Production Center



In this Example, the sequence $F' E' D'$ is a promoter, the sequence $G H J K$ is an Anti-Sense sequence and $X Y Z$ is a Poly A signal

Figure 38
Continuation of process from Figure 37

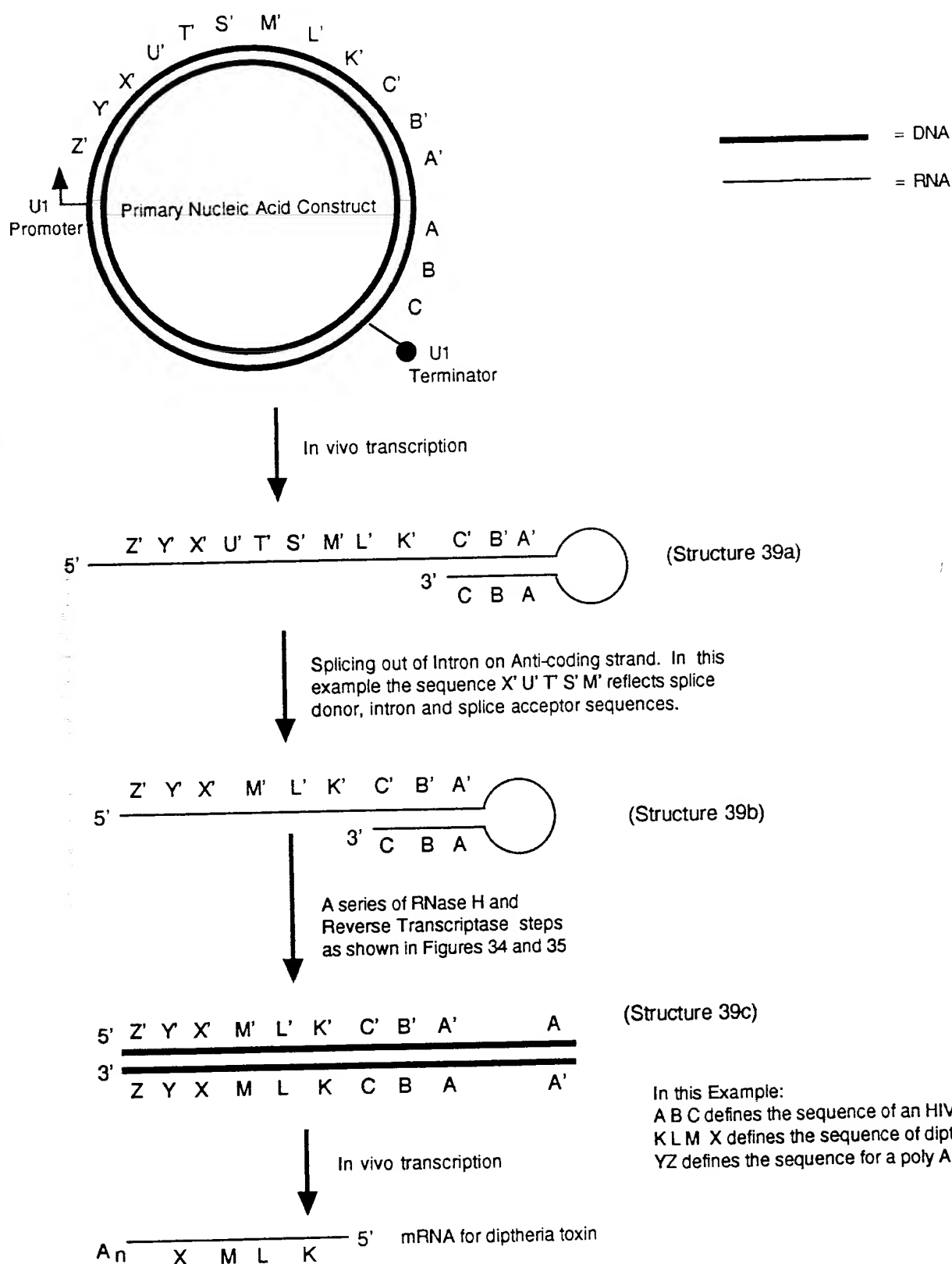


Figure 39

Construct which propagates a Production Center capable of Inducible Suicide

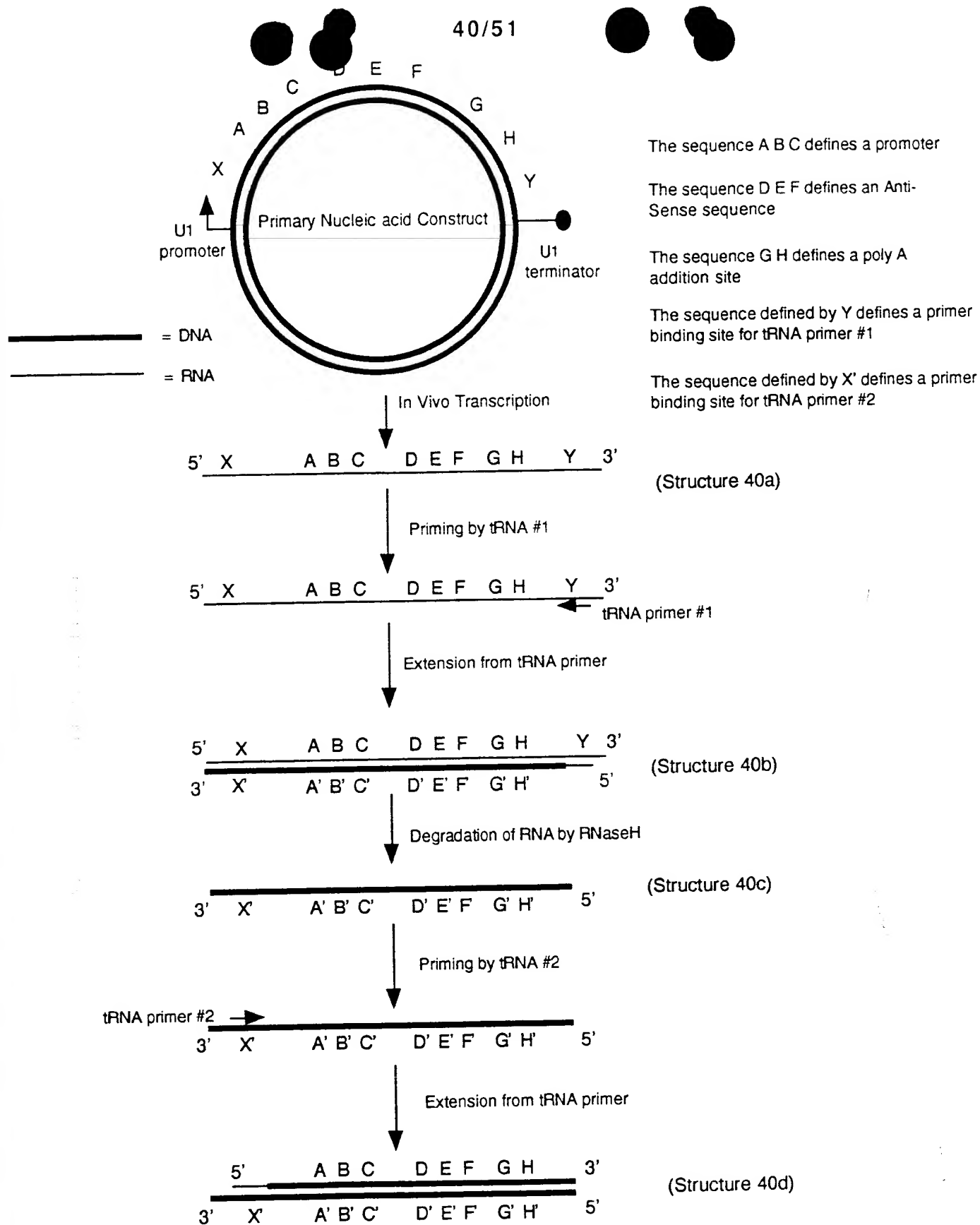


Figure 40

Use of tRNA primers to create a DNA construct for secondary production of transcripts

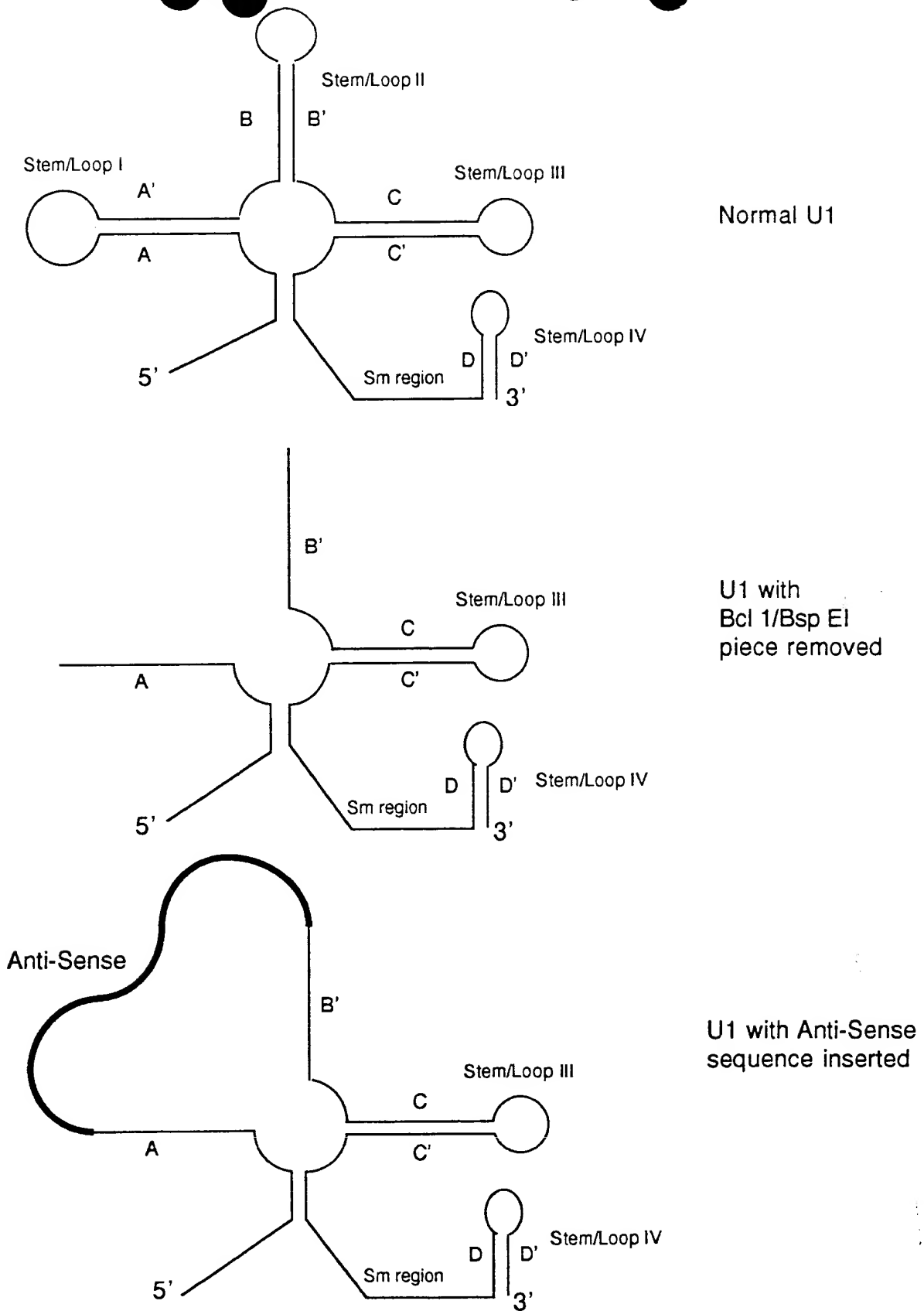


Figure 41

Excision of Sequences from U1 Transcript Region
and Replacement with Novel Sequences

(A) Anti-sense oligomers

HVA-1 GAT CCG GAT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT

HVA-2 CCG GAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGC TTA AGC CTC AAT CCG

HVB-1 GAT CCG GAC CTT GAG GAG GTC TTC GTC GCT GTC TCC GCT TCT TCC TGC CAT AGG AGA GCC TAA GGT

HVB-2 CCG GAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA GGT CCG

HVC-1 GAT CCG GAT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT

HVC-2 CCG GAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC ATC CG

HVD-1 GAT CAG CAT GCC TGC AGG TCG ACT CTA GAC CCG GGT ACC GAG CTC GCC CTA TAG TGA GT C GTA TTA T

HVD-2 CCG GAT AAT ACG ACT CAC TAT AGG GCG AGC TCG GTA CCC GGG TCT AGA GTC GAC CTG CAG GCA TGC T

(B) Replacement of U1 sequences with HIV Anti-sense sequences

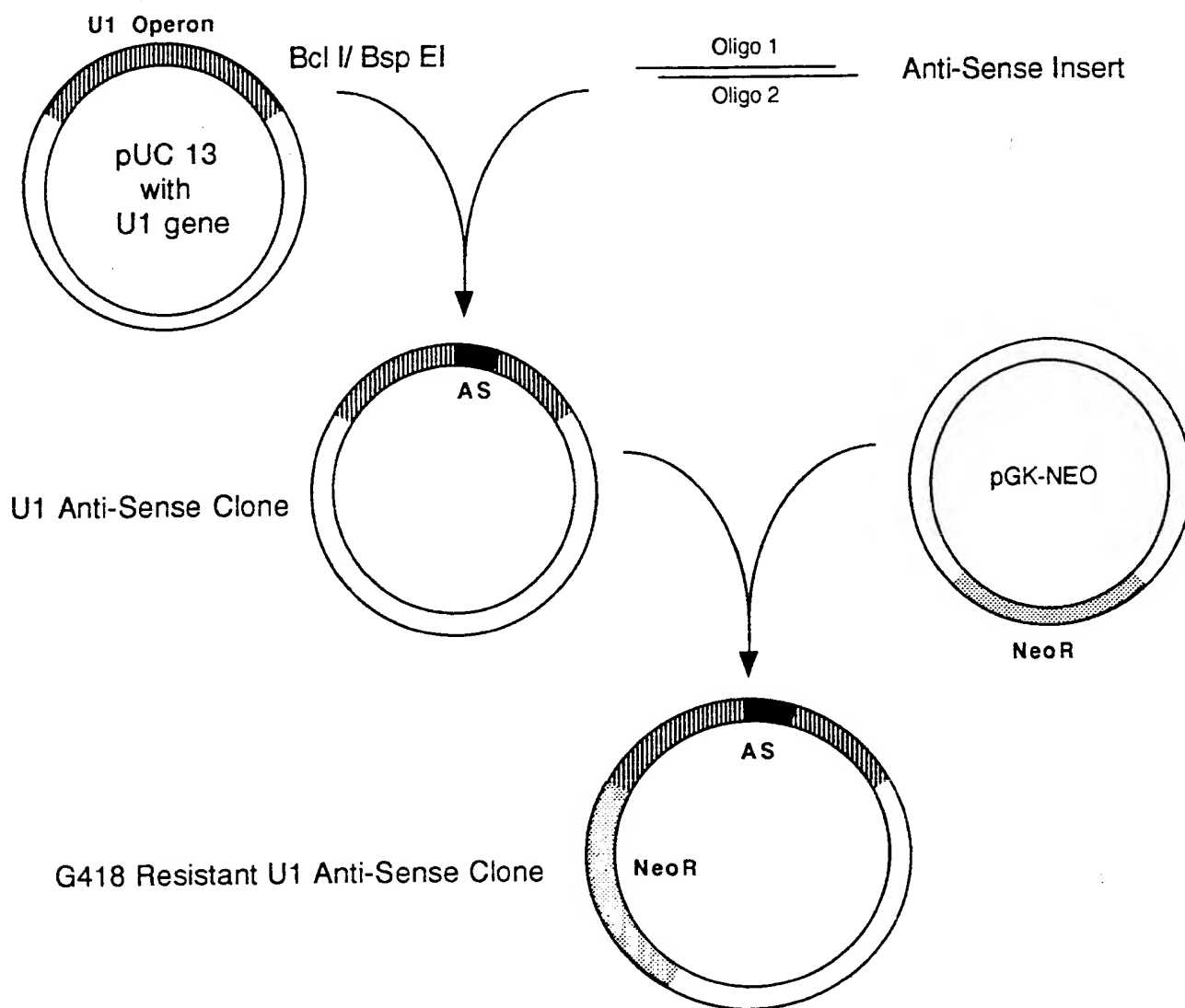


Figure 42

Insertion of Anti-Sense Sequences into U1 Operons

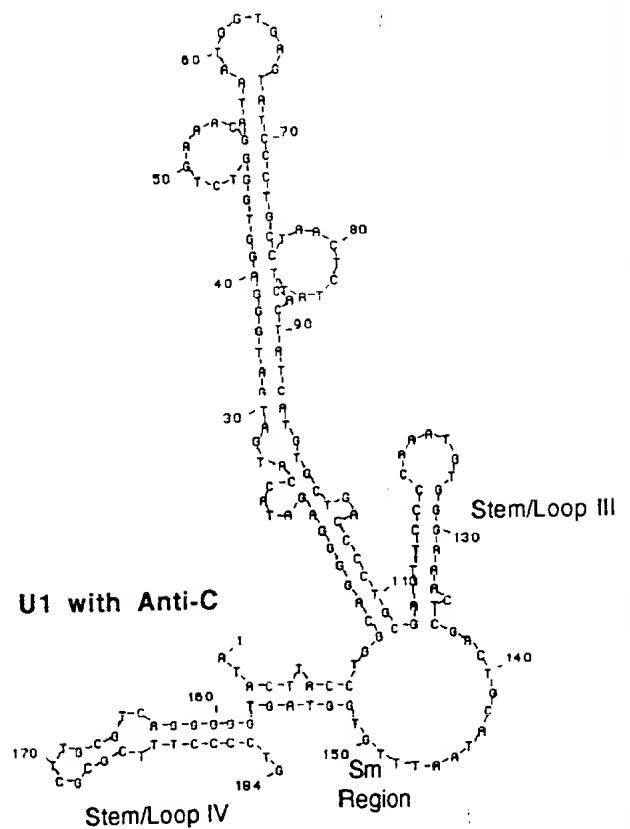
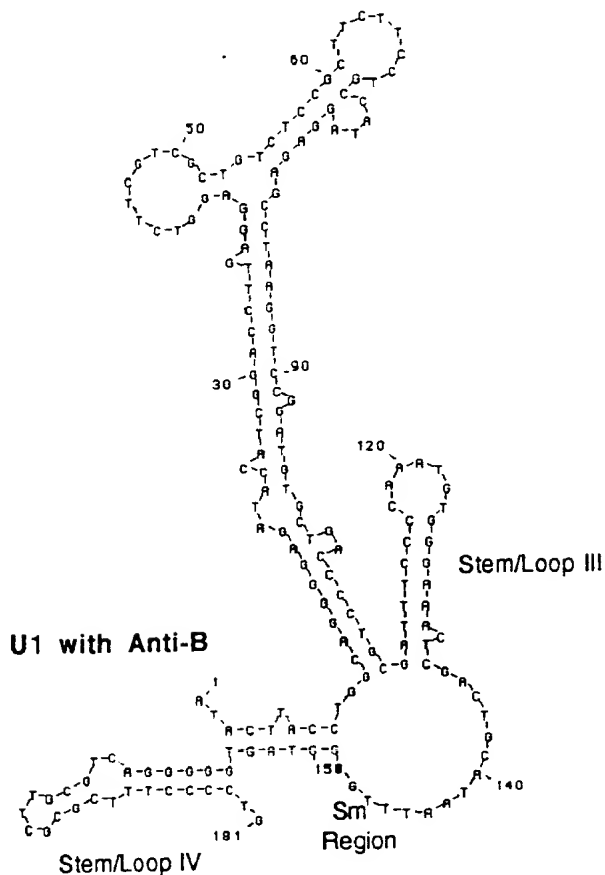
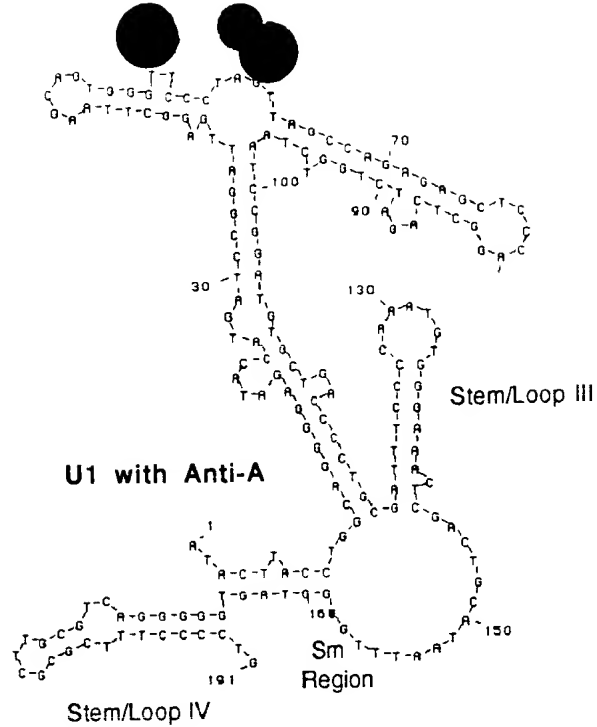
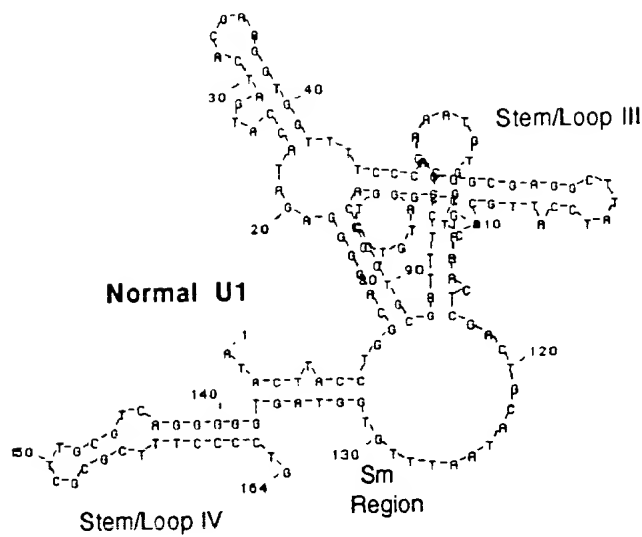


Figure 43

Predicted Secondary structures for U1
Transcripts with Anti-sense Substitutions

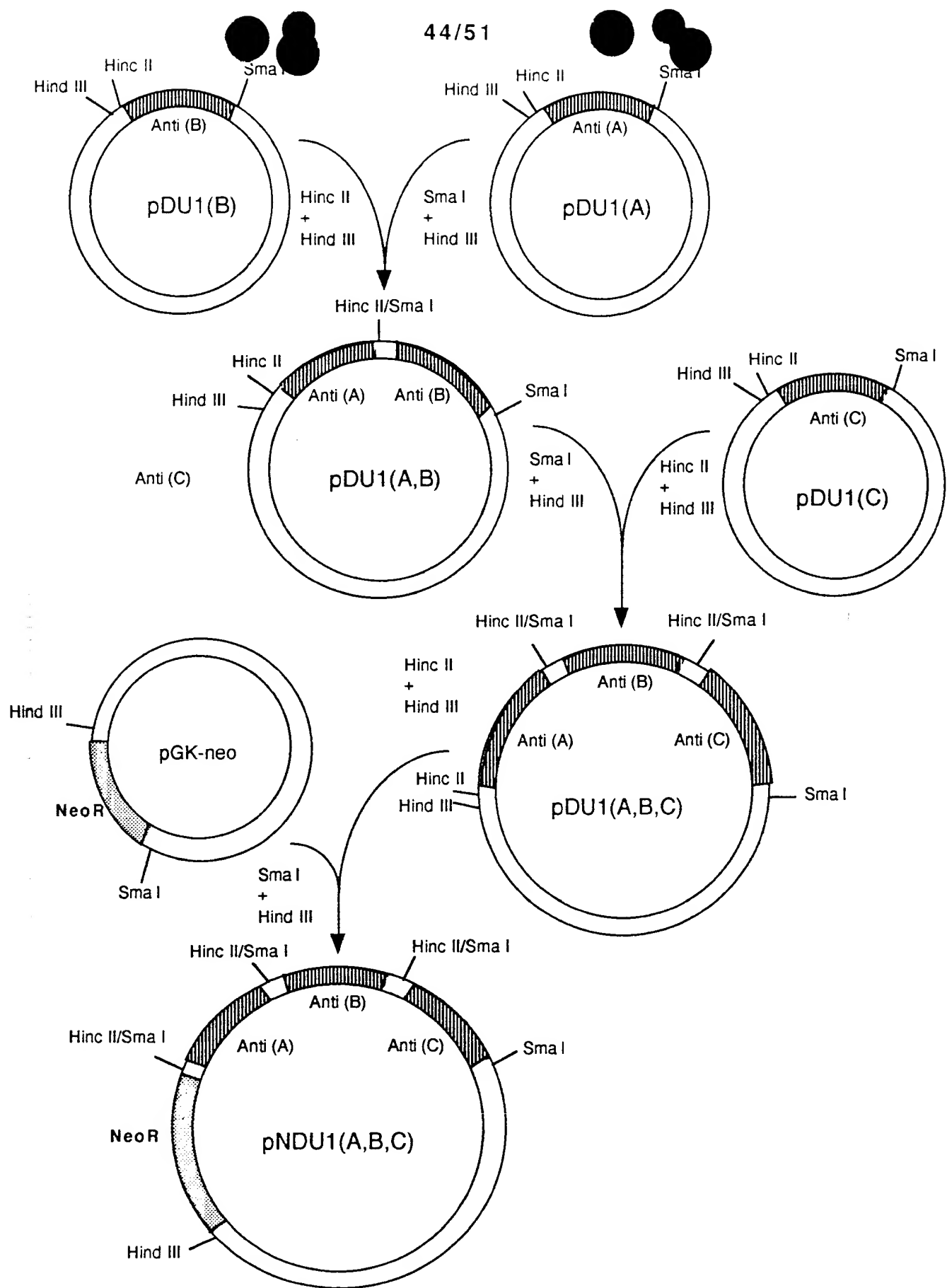


Figure 44
Construction of U1 Multiple Operon Clone

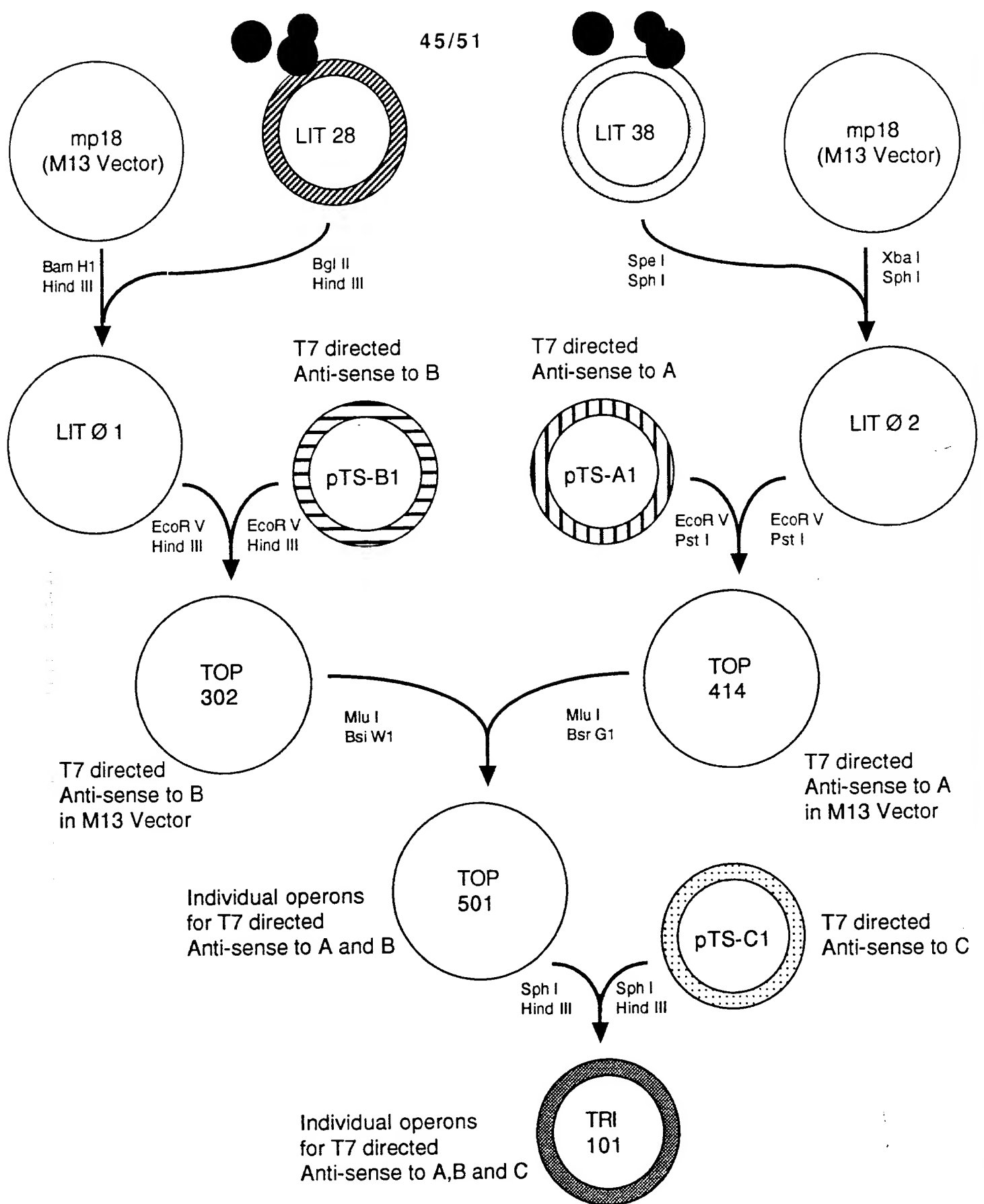
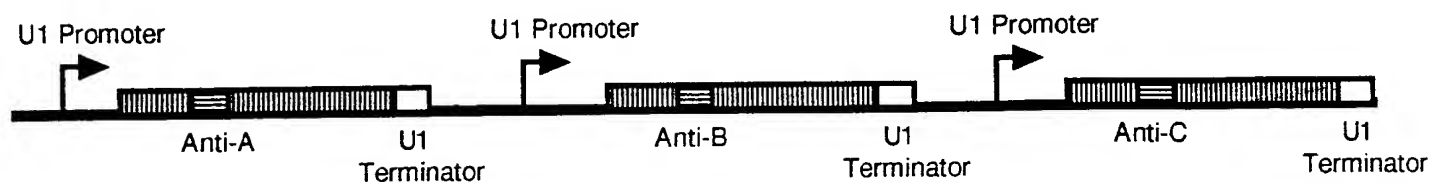


Figure 45
Construction of T7 Triple Operon

pNDU1(A,B,C)

Triple U1 Operon Construct with HIV Anti-Sense



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Triple T7 Operon Construct with HIV Anti-Sense

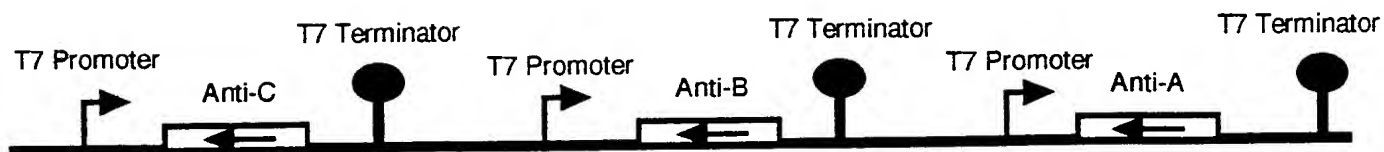


Figure 46

Structures of Triple Operon Constructs
from Figures 44 and 45

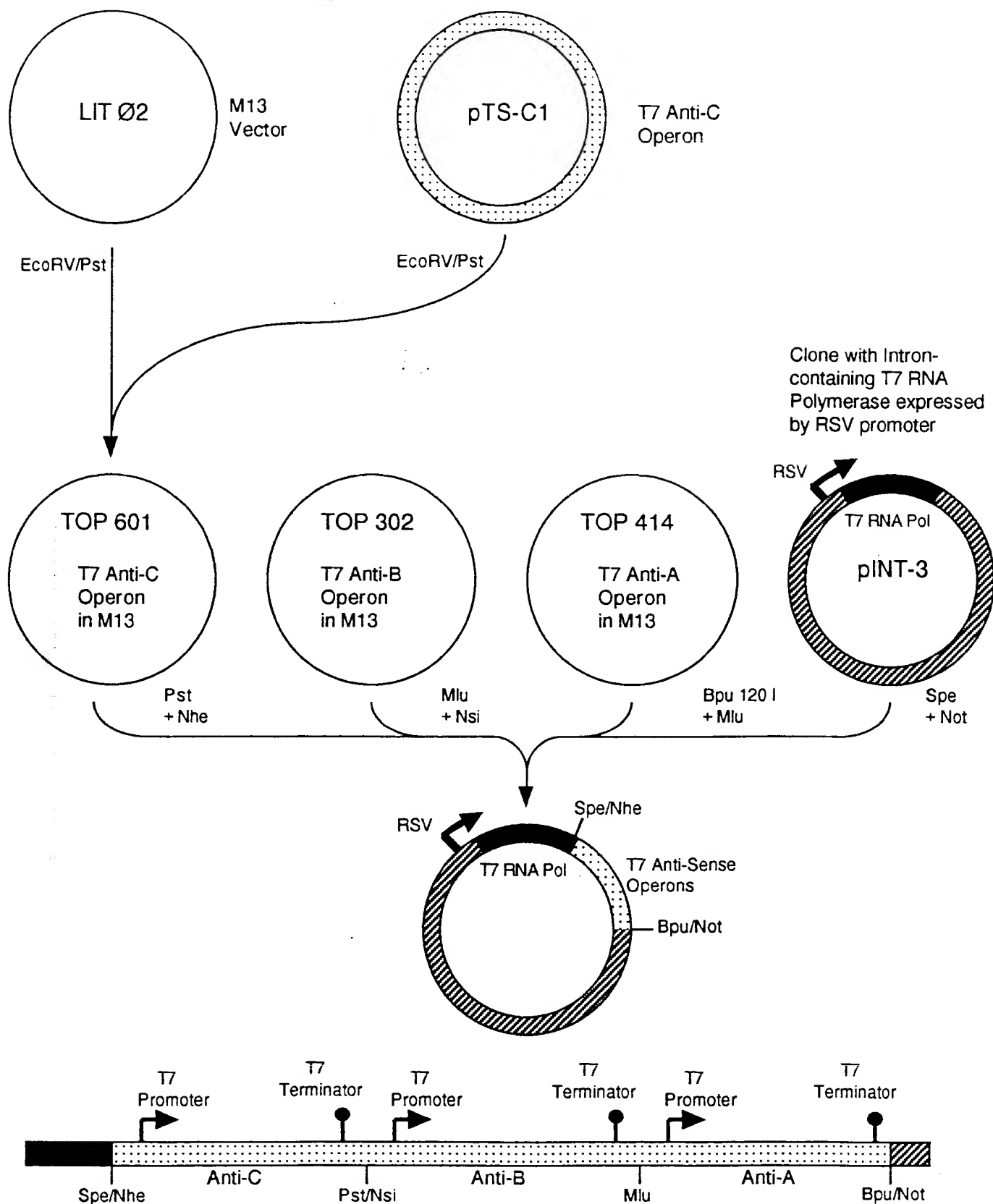
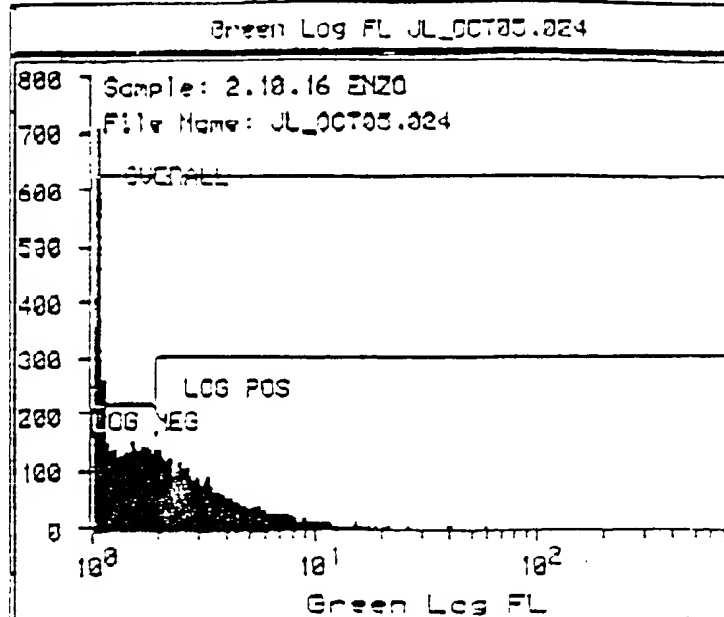
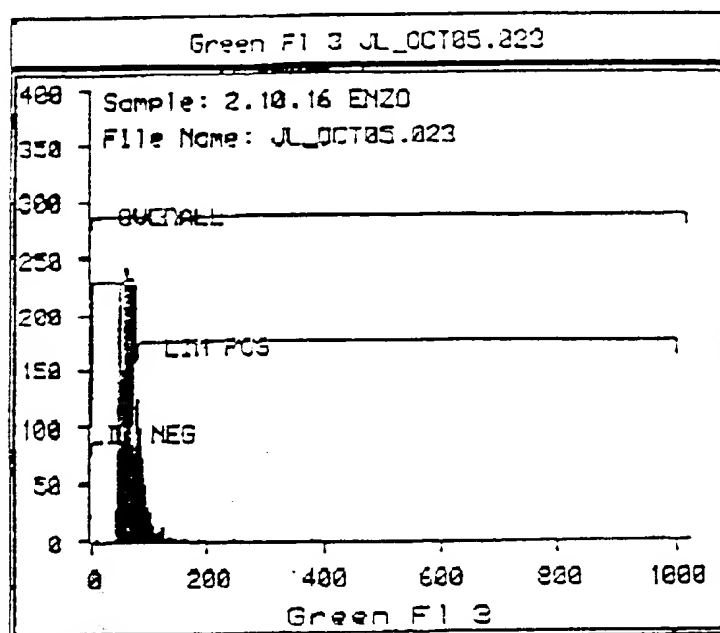


Figure 47
Construction of Multiple T7 Operons in
Vector coding for T7 RNA Polymerase



Global Statistics									
1. Green F1 3 JL_OCT05.023				Total = 7509					
2. Green Log FL JL_OCT05.024				Total = 7509					
Hist	Region	Bounds	Counts	%	Mean X	Mean Y	Mode	Xc	
1.	LIN NEG	1 78	5714	76.1	63.65		70	14	
	LIN POS	85 1002	1129	15.0	97.34		85	17	
	OVERALL	1 1024	7509	100.0	70.28		70	23	
2.	LOG NEG	2 2	4211	56.1	2.34		2	21	
	LOG POS	2 1001	3407	45.4	4.76		3	69	
	OVERALL	2 1001	7509	100.0	3.43		2	88	

Figure 48

Flow cytometry data measuring binding of
anti-CD4+ antibody to HIV resistant U037 cells

49.51



Figure 49

PCR amplification of gag region
indicating absence of HIV in
viral resistant cell line (2.10.16)
after challenge

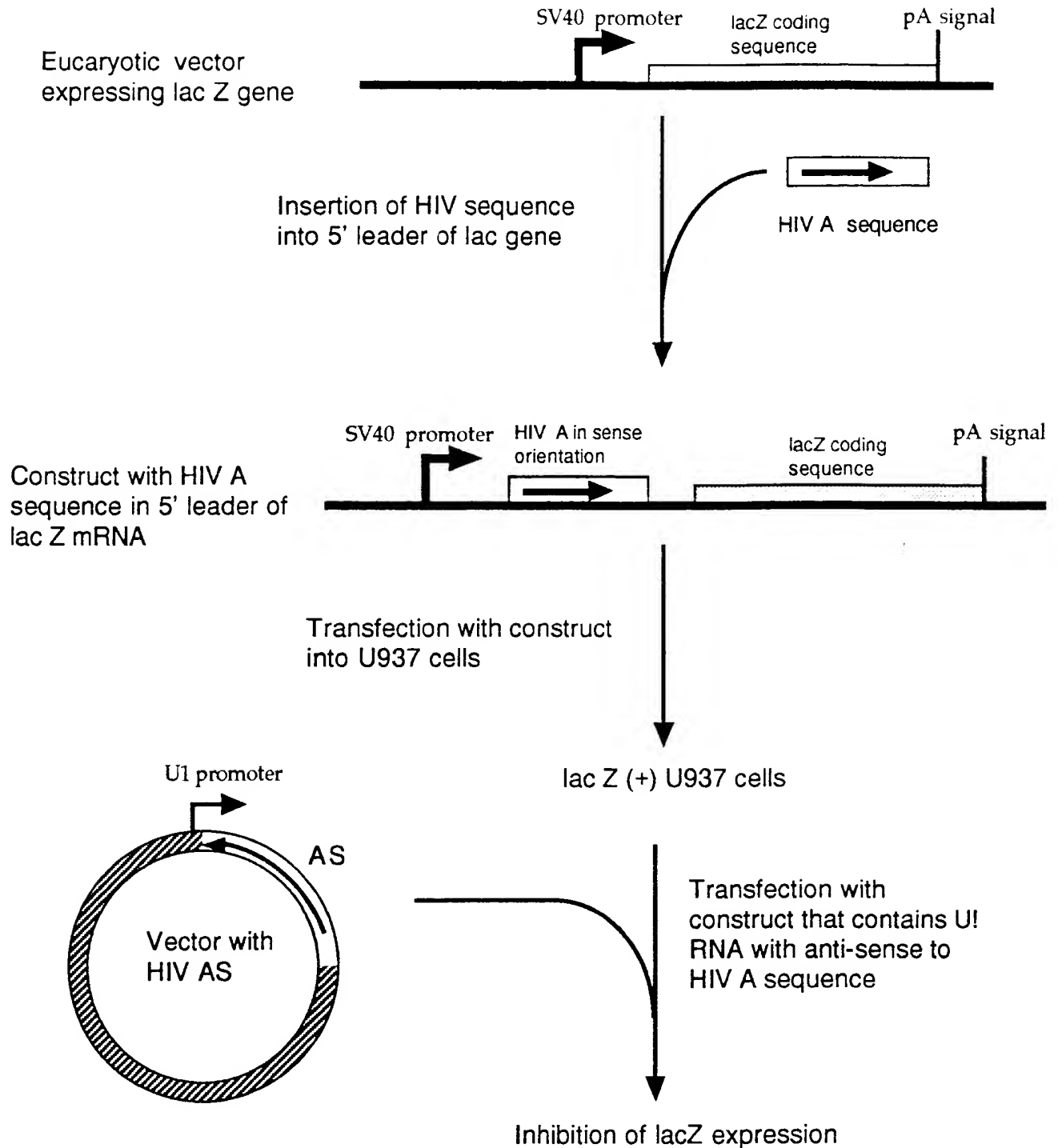


Figure 50

Clone with target-lacZ fusion will have reduced expression of lacZ after transfection by HIV Anti-sense construct

Enzyme activity as expressed by A ₄₂₀ readings in extracts prepared from			
	2.5 x 10 ⁴ cells	5 x 10 ⁴ cells	1.0 x 10 ⁵ cells
U 937 [untransfected]	0.018	0.023	0.034
U 937 [HIV A clone]	0.154	0.277	0.566
U937 [HIV A / Anti-A]	0.010	0.017	0.027
U 937 [HIV A/Anti-ABC]	0.013	0.021	0.035
U 937 [HIV A / Null DNA]	0.120	0.212	0.337

[B] Expression of Beta-galactosidase activity by *In situ* assay :

U 937 [untransfected] no blue spots in cells
 U 937 [HIV A clone] blue spots in cells
 U 937 [HIV A/Anti A] no blue spots in cells
 U 937 [HIV A/Anti ABC] no blue spots in cells
 U 937 [HIV A / Null DNA] blue spots in cells

Figure 51

Expression of Beta-galactosidase activity
in extracts